Attorney Docket No. 029318-0973

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicants: H. William Bosch et al.

Title: NOVEL NIMESULIDE COMPOSITIONS

Appl. No.: 10/697,703

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Examiner: Sara CLARK

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BRIEF ON APPEAL

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In re Kubin

In re Marek Z. KUBIN and Raymond G. Goodwin.

No. 2008–1184.

United States Court of Appeals, Federal Circuit.

April 3, 2009.

Background: Applicants for patents related to claimed biotechnology invention for isolating and sequencing of human gene that encoded particular domain of protein, specifically DNA molecules, or polynucleotides, encoding polypeptide known as Natural Killer Cell Activation Inducing Ligand (NAIL). The United States Patent and Trademark Office, Board of Patent Appeals and Interferences, 2007 WL 2070495, rejected claims as obvious and invalid for lack of written description. Applicants appealed.

Holding: The Court of Appeals, Rader, Circuit Judge, held that claimed gene sequence was unpatentably obvious in light of abundant prior art.

Affirmed.

1. Patents ⇐113(6)

Court of Appeals reviews factual findings by the Board of Patent Appeals and Interferences for lack of substantial evidence, and the Board's legal conclusions without deference.

2. Patents ⇐16.13

In determining patentability, obviousness of a claimed invention is a question of law based on underlying findings of fact. 35 U.S.C.A. § 103.

3. Patents ⇐16(2, 3), 36.1(1)

An analysis of obviousness to determine patentability must be based on several factual inquiries: (1) the scope and content of the prior art, (2) the differences between the prior art and the claims at issue, (3) the level of ordinary skill in the art at the time the invention was made, and (4) objective evidence of nonobviousness, if any. 35 U.S.C.A. § 103.

4. Patents ⇐16.13

The teachings of a prior art reference are underlying factual questions in the obviousness inquiry for patentability of a claimed invention. 35 U.S.C.A. § 103.

5. Patents ⇐36(3)

Board of Patent Appeals and Interferences' conclusion, in rejecting claims of patent application for isolating human gene sequence for natural killer cell activation inducing ligand (NAIL), that claimed sequence was obvious in light of abundant prior art, was supported by substantial evidence including that application disclosed use of standard biochemical methods outlined in prior art to isolate gene sequence for NAIL, that researcher of ordinary skill in art would have recognized that prior art discussed detailed protocol for identifying, isolating, and cloning equivalent of NAIL, that prior art did not teach away from combining its teachings with other references regarding gene sequence, and that skilled artisan would have had resoundingly reasonable expectation of success in deriving claimed invention in light of teachings of prior art. 35 U.S.C.A. § 103(a).

6. Patents ⇐16.5(1)

A prior art reference may be said to "teach away" when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the patent applicant. 35 U.S.C.A. § 103.

See publication Words and Phrases for other judicial constructions and definitions.

Patents ⇐328(2)

5,688,690. Cited as Prior Art.

Barbara R. Rudolph, Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., of Washington, DC, argued for appellants. With her on the brief were Herbert H. Mintz and Bart A. Gerstenblith. Of counsel was Stuart L. Watt, Wendy A. Whiteford and Gail A. Katz, Amgen Inc., of Thousand Oaks, CA, and Kathleen Fowler, of Seattle, WA.

Janet A. Gongola, Associate Solicitor, Office of the Solicitor, United States Patent and Trademark Office, of Arlington, VA, argued for the Director of the United States Patent and Trademark Office. With her on the brief were William G. Jenks, Mary L. Kelly, and Stephen Walsh, Associate Solicitors. Of counsel was Raymond T. Chen, Associate Solicitor.

Rouget F. Henschel, Foley & Lardner LLP, of Washington, DC, for amicus curiae Biotechnology Industry Organization. With him on the brief were Stephen B. Maebius and Philip G. Kiko. Of counsel was Hans Sauer, Biotechnology Industry Organization, of Washington, DC, and Brian P. Barrett, Eli Lilly and Company, of Indianapolis, IN.

Matthew I. Kreeger, Morrison & Foerster LLP, of San Francisco, CA, for amicus curiae Novartis Vaccines and Diagnostics, Inc. Of counsel on the brief was Beth S. Brinkmann, of Washington, DC.

Jeffrey P. Kushan, Sidley Austin LLP, of Washington, DC, for amici curiae Glaxosmithkline, et al. With him on the brief were Paul J. Zegger, Jon P. Santamauro, and Eric M. Solovy.

James J. Kelley, Eli Lilly and Company, of Indianapolis, IN, for amicus curiae Eli Lilly and Company. With him on the brief were MaryAnn Wiskerchen, Gregory A. Cox, Steven P. Caltrider and Robert A. Armitage.

Before RADER, FRIEDMAN, and LINN, Circuit Judges.

RADER, Circuit Judge.

Marek Kubin and Raymond Goodwin ("appellants") appeal from a decision of the Board of Patent Appeals and Interferences (the "Board") rejecting the claims of U.S. Patent Application Serial No. 09/667,859 ("'859 Application") as obvious under 35 U.S.C. § 103(a) and invalid under 35 U.S.C. § 112 ¶ 1 for lack of written description. *Ex parte Kubin*, No. 2007-0819, 83 U.S.P.Q.2d 1410 (B.P.A.I.2007) ("*Board Decision*"). Because the Board correctly determined that appellants' claims are unpatentably obvious, this court affirms.

I.

This case presents a claim to a classic biotechnology invention—the isolation and sequencing of a human gene that encodes a particular domain of a protein. This court provided a primer on the basics of this technology in *In re O'Farrell*, 853 F.2d 894, 895–99 (Fed.Cir.1988). Specifically, appellants claim DNA molecules ("polynucleotides") encoding a protein ("polypeptide") known as the Natural Killer Cell Activation Inducing Ligand ("NAIL").

Natural Killer ("NK") cells, thought to originate in the bone marrow, are a class of cytotoxic lymphocytes that play a major role in fighting tumors and viruses. NK cells express a number of surface molecules which, when stimulated, can activate cytotoxic mechanisms. NAIL is a specific receptor protein on the cell surface that plays a role in activating the NK cells.

The specification of the claimed invention recites an amino acid sequence of a NAIL polypeptide. The invention further isolates and sequences a polynucleotide that encodes a NAIL polypeptide. Moreover, the inventors trumpet their alleged discovery of a binding relationship between NAIL and a protein known as CD48. The NAIL–CD48 interaction has

important biological consequences for NK cells, including an increase in cell cytotoxicity and in production of interferon. Representative claim 73 of appellants' application claims the DNA that encodes the CD48-binding region of NAIL proteins:

> 73. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide at least 80% identical to amino acids 22-221 of SEQ ID NO:2, wherein the polypeptide binds CD48.

In other words, appellants claim a genus of isolated polynucleotides encoding a protein that binds CD48 and is at least 80% identical to amino acids 22-221 of SEQ ID NO:2—the disclosed amino acid sequence for the CD48-binding region of NAIL.

Appellants' specification discloses nucleotide sequences for two polynucleotides falling within the scope of the claimed genus, namely SEQ ID NO:1 and SEQ ID NO:3. SEQ ID NO: 1 recites the specific coding sequence of NAIL, whereas SEQ ID NO: 3 recites the full NAIL gene, including upstream and downstream noncoding sequences. The specification also contemplates variants of NAIL that retain the same binding properties:

> Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.
>
> A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, e.g., involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

'859 Application at 26. However, the specification does not indicate any example variants of NAIL that make these conservative amino acid substitutions.

II.

The Board rejected appellants' claims as invalid under both § 103 and § 112. With regard to the § 112 rejection, the Board found the genus of nucleic acids of representative claim 73 unsupported by an adequate written description. First, the Board observed that although appellants had sequenced two nucleic acids falling within the scope of claim 73, they had not disclosed any variant species where amino acids 22-221 were different in any way from the disclosed SEQ ID NO:2 sequence. Thus, the Board concluded that appellants were not entitled to their genus claim of DNA molecules encoding proteins 80% identical to SEQ ID NO:2:

> [Appellants] have not described what domains of those sequences are correlated with the required binding to CD48, and thus have not described which of NAIL's amino acids can be varied and still maintain binding. Thus ... their Specification would not have shown possession of a sufficient number of sequences falling within their potentially large genus to establish possession of their claimed genus.
>
> Without a correlation between structure and function, the claim does little more than define the claimed invention by

function. That is not sufficient to satisfy the written description requirement.

Board Decision at 16–17.

Regarding obviousness, the Board rejected appellants' claims over the combined teachings of U.S. Patent No. 5,688,-690 ("Valiante") and 2 Joseph Sambrook et al., *Molecular Cloning: A Laboratory Manual* 43–84 (2d ed.1989) ("Sambrook"). The Board also considered, but found to be cumulative to Valiante and Sambrook, Porunelloor Mathew et al., *Cloning and Characterization of the 2B4 Gene Encoding a Molecule Associated with Non-MHC-Restricted Killing Mediated by Activated Natural Killer Cells and T Cells,* 151 J. Immunology 5328–37 (1993) ("Mathew").

Valiante discloses a receptor protein called "p38" that is found on the surface of human NK cells. Valiante teaches that the p38 receptor is present on virtually all human NK cells and "can serve as an activation marker for cytotoxic NK cells." '690 Patent col.3 ll.3–4; *see also id.* at col.5 ll.6–7 ("Stimulation of p38 results in activation of cytotoxicity"). Valiante also discloses and claims a monoclonal antibody specific for p38 called "mAB C1.7." The Board found (and appellants do not dispute) that Valiante's p38 protein is the same protein as NAIL. *Board Decision* at 4. A monoclonal antibody is an antibody that is mass produced in the laboratory from a single clone and that recognizes only one antigen. Monoclonal antibodies are useful as probes for specifically identifying and targeting a particular kind of cell.

Valiante teaches that "[t]he DNA and protein sequences for the receptor p38 may be obtained by resort to conventional methodologies known to one of skill in the art." '690 Patent col.7 ll.49–51.

> For example, the receptor may be isolated by immunoprecipitation using the mAb C1.7. Alternatively, the receptor may be obtained by prokaryotic expression cloning, using the lambda phage gtll, which is described in detail in Sambrook et al, Molecular Cloning, A Laboratory Manual, 2d edit., Cold Spring Harbor, N.Y. (1989), pp. 2.43–2.84, incorporated by reference herein.
>
> Additionally, as described in Example 12 below, the DNA sequence encoding the receptor can be obtained by the "panning" technique of screening a human NK cell library by eukaryotic expression cloning, of which several are known. Briefly, plasmids are constructed containing random sequences of a human NK cell library which are obtained by restriction digestion. Such libraries may be made by conventional techniques or may be available commercially.
>
> Suitable cells, preferably mammalian cells, such as COS–1 cells, are transfected with the plasmids and the mAb C1.7 antibody employed to identify transfectants containing the receptor after repeated rounds of panning. The receptor insert in these cells is then identified and sequenced by conventional techniques, such as overlapping deletion fragments [Sambrook et al. cited above]. Other known techniques may also be employed to sequence the receptor and/or the mAb C1.7.

Id. at col.7 l.51–col.8 l.7. Example 12 of Valiante's patent further describes a five-step cloning protocol for "isolating and identifying the p38 receptor." *Id.* at col.18 l.6–col.19 l.28. Valiante discloses neither the amino acid sequence of p38 recognized by mAb C1.7 nor the polynucleotide sequence that encodes p38. Sambrook, incorporated by reference (as cited above) in Valiante, describes methods for molecular cloning. Sambrook does not discuss how to clone any particular gene, but provides detailed instructions on cloning materials and techniques.

The Mathew reference discloses a cell surface receptor protein called 2B4 "expressed on all NK ... cells." Mathew at 5328. Mathew discloses that 2B4 is involved in activating mouse NK cells, and further teaches the "chromosomal mapping, cloning, expression, and molecular characterization of the 2B4 gene." *Id.* at 5329. Further, Mathew teaches a monoclonal antibody, mAb 2B4, specific to 2B4, and a detailed cloning protocol for obtaining the sequence of the gene that codes for the 2B4 protein. *Id.* at 5328–330. The Board found that Mathew's signaling molecule 2B4 is the murine (mouse) version of Valiante's p38. *Board Decision* at 5. The Board viewed Mathew's teachings to be "cumulative to the teachings in Valiante and Sambrook and merely ... exemplary of how routine skill in the art can be utilized to clone and sequence the cDNA of a similar polypeptide." *Id.*

The Board found as a factual matter that appellants used conventional techniques "such as those outlined in Sambrook" to isolate and sequence the gene that codes for NAIL. *Id.* The Board also found that appellants' claimed DNA sequence is "isolated from a cDNA library ... using the commercial monoclonal antibody C1.7 ... disclosed by Valiante." *Id.* With regard to the amino acid sequence referred to as SEQ ID NO:2, the Board found that

> Valiante's disclosure of the polypeptide p38, and a detailed method of isolating its DNA, including disclosure of a specific probe to do so, i.e., mAb C1.7, established Valiante's possession of p38's amino acid sequence and provided a reasonable expectation of success in obtaining a polynucleotide encoding p38, a polynucleotide within the scope of Appellants' claim 73. (See Valiante, col.7, l.48 to col.8, l.7.)

Id. at 6. Because of NAIL's important role in the human immune response, the Board further found that "one of ordinary skill in the art would have recognized the value of isolating NAIL cDNA, and would have been motivated to apply conventional methodologies, such as those disclosed in Sambrook and utilized in Valiante, to do so." *Id.* at 6–7.

Based on these factual findings, the Board turned to the legal question of obviousness under § 103. Invoking the Supreme Court's decision in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 127 S.Ct. 1727, 167 L.Ed.2d 705 (2007), the Board concluded that appellants' claim was " 'the product not of innovation but of ordinary skill and common sense,' leading us to conclude NAIL cDNA is not patentable as it would have been obvious to isolate it." *Board Decision* at 9 (citing *KSR*, 550 U.S. at 421, 127 S.Ct. 1727).

Appellants appeal the Board's decisions both as to obviousness and written description. This court has jurisdiction under 28 U.S.C. § 1295(a)(4) and 35 U.S.C. § 141.

III.

[1] This court reviews the Board's factual findings for lack of substantial evidence, and its legal conclusions without deference. *In re Gartside*, 203 F.3d 1305, 1315 (Fed.Cir.2000).

[2–4] Obviousness is a question of law based on underlying findings of fact. An analysis of obviousness must be based on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art at the time the invention was made; and (4) objective evidence of nonobviousness, if any. *See Graham v. John Deere Co.*, 383 U.S. 1, 17–18, 86 S.Ct. 684, 15 L.Ed.2d 545 (1966). The teachings of a prior art reference are underlying factual questions in the obviousness inquiry. *See*

Para–Ordnance Mfg., Inc. v. SGS Imp. Int'l, Inc., 73 F.3d 1085, 1088 (Fed.Cir. 1995).

A.

As a factual matter, the Board concluded that appellants' methodology of isolating NAIL DNA was essentially the same as the methodologies and teachings of Valiante and Sambrook. Appellants charge that the record does not contain substantial evidence to support this Board conclusion.

This emphasis on similarities or differences in methods of deriving the NAIL DNA misses the main point of this obviousness question. Of note, the record nowhere suggests that the technique in Valiante's Example 12 for isolating NAIL (p38) DNA, even if slightly different than the technique disclosed in the claimed invention, would not yield the same polynucleotide claimed in claim 73. Stated directly, the record shows repeatedly that Valiante's Example 12 produces for any person of ordinary skill in this art the claimed polynucleotide.

More to the point, however, any putative difference in Valiante's/Sambrook's and appellants' *processes* does not directly address the obviousness of representative claim 73, which claims a genus of *polynucleotides.* The difference between Valiante's and the application's techniques might be directly relevant to obviousness in this case if Kubin and Goodwin had claimed a method of DNA cloning or isolation. But they did not. Appellants claim a gene sequence. Accordingly, the obviousness inquiry requires this court to review the Board's decision that the claimed sequence, not appellants' unclaimed cloning technique, is obvious in light of the abundant prior art.

[5] In any event, this court determines that the Board had substantial evidence to conclude that appellants used conventional techniques, as taught in Valiante and Sambrook, to isolate a gene sequence for NAIL. In particular, appellants' arguments that Valiante and Sambrook are deficient because they do not provide "any guidance for the preparation of cell culture that will serve as a useful source of mRNA for the preparation of a cDNA library," Appellants' Br. 34, are diminished by appellants' own disclosure:

> A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by *standard biochemical methods (such as those outlined in Sambrook* et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989)).

'859 Application at 16–17 (emphasis added). Thus, Kubin and Goodwin cannot represent to the public that their claimed gene sequence can be derived and isolated by "standard biochemical methods" discussed in a well-known manual on cloning techniques, while at the same time discounting the relevance of that very manual to the obviousness of their claims. For this reason as well, substantial evidence supports the Board's factual finding that "[a]ppellants employed conventional methods, 'such as those outlined in Sambrook,' to isolate a cDNA encoding NAIL and determine the cDNA's full nucleotide sequence (SEQ NOS: 1 & 3)." *Board Decision* at 5.

In a similar vein, this court reviews the Board's reference to the teachings of Mathew and the connection between Mathew's 2B4 and Valiante's p38 proteins.

As an initial point, the Board referenced Mathew only as cumulative of Sambrook and Valiante. Therefore, the Board's obviousness analysis does not explicitly rely on Mathew at all. Instead the Board observed that Mathew is "exemplary of how routine skill in the art can be utilized to clone and sequence the cDNA of a similar polypeptide." *Id.* In that connection, the record shows that a researcher of ordinary skill in this art would have recognized that both Valiante and Mathew are indisputably focused on regulation of NK cells—Mathew with regard to mice and Valiante with regard to humans. Like Valiante's Example 12, Mathew discusses a detailed protocol for identifying, isolating, and cloning cDNA encoding 2B4, which was later discovered to be the murine equivalent of Valiante's p38 and appellants' NAIL protein. Moreover, Mathew expressly states that his genomic DNA blot analysis "identified a human homologue of the 2B4 gene." Mathew at 5333. In sum, substantial evidence supports the Board's conclusion that Matthew reinforces the relative ease of deriving the claimed sequence following the teachings of the prior art.

[6] This court notes that Matthew contains some data that "suggests that [the] 2B4 gene is not expressed in humans." *Id.* This part of the record, however, does not undermine the Board's correct conclusion that Mathew does not "teach away" from combining its teachings with Valiante. "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley,* 27 F.3d 551, 553 (Fed.Cir.1994). According to Mathew, "[i]t appears ... that the 2B4 gene is somewhat conserved during evolution." Mathew at 5335. Mathew's quasi-agnostic stance toward the existence of a human homologue of the 2B4 gene cannot fairly be seen as dissuading one of ordinary skill in the art from combining Mathew's teachings with those of Valiante. Rather, Mathew's disclosure, in light of Valiante's teachings regarding the p38 protein and its role in NK cell activation, would have aroused a skilled artisan's curiosity to isolate the gene coding for p38. Thus, the record supplies ample evidence to support the Board's finding that Mathew "exemplifies how the cDNA encoding 2B4, the mouse version of Valiante's p38 expressed on all NK cells, can be isolated and sequenced." *Board Decision* at 10.

This court also observes that the Board had no obligation to predicate its obviousness finding on factual findings regarding a prior art teaching of NAIL's binding to the CD48 protein. Even if no prior art of record explicitly discusses the "wherein the polypeptide binds CD48" aspect of claim 73, the Kubin–Goodwin application itself instructs that CD48 binding is not an additional requirement imposed by the claims on the NAIL protein, but rather a property necessarily present in NAIL. *See, e.g.,* '859 Application at 1, 8 (describing CD48 as NAIL's "counterstructure"). Because this court sustains, under substantial evidence review, the Board's finding that Valiante's p38 is the same protein as appellant's NAIL, Valiante's teaching to obtain cDNA encoding p38 also necessarily teaches one to obtain cDNA of NAIL that exhibits the CD48 binding property. *See, e.g., Gen. Elec. Co. v. Jewel Incandescent Lamp Co.,* 326 U.S. 242, 249, 66 S.Ct. 81, 90 L.Ed. 43 (1945) ("It is not invention to perceive that the product which others had discovered had qualities they failed to detect."); *In re Wiseman,* 596 F.2d 1019, 1023 (CCPA 1979) (rejecting the notion that "a structure suggested by the prior art, and, hence, potentially in the possession of the public, is patentable ... because it also possesses an inherent, but hitherto unknown, function which [paten-

tees] claim to have discovered. This is not the law. A patent on such a structure would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art.").

B.

The instant case also requires this court to consider the Board's application of this court's early assessment of obviousness in the context of classical biotechnological inventions, specifically *In re Deuel*, 51 F.3d 1552 (Fed.Cir.1995). In *Deuel*, this court reversed the Board's conclusion that a prior art reference teaching a method of gene cloning, together with a reference disclosing a partial amino acid sequence of a protein, rendered DNA molecules encoding the protein obvious. *Id.* at 1559. In reversing the Board, this court in *Deuel* held that "knowledge of a protein does not give one a conception of a particular DNA encoding it." *Id.* Further, this court stated that "obvious to try" is an inappropriate test for obviousness.

> *[T]he existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious,* in the absence of other prior art that suggests the claimed DNAs.... *"Obvious to try" has long been held not to constitute obviousness.* A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out.

Id. (internal citations omitted) (emphases added). Thus, this court must examine *Deuel*'s effect on the Board's conclusion that Valiante's teaching of the NAIL protein, combined with Valiante's/Sam brook's teaching of a method to isolate the gene sequence that codes for NAIL, renders claim 73 obvious.

With regard to *Deuel*, the Board addressed directly its application in this case. In particular, the Board observed that the Supreme Court in *KSR* cast doubts on this court's application of the "obvious to try" doctrine:

> To the extent *Deuel* is considered relevant to this case, we note the Supreme Court recently cast doubt on the viability of *Deuel* to the extent the Federal Circuit rejected an "obvious to try" test. *See KSR Int'l Co. v. Teleflex Inc.*, [550 U.S. 398], 127 S.Ct. 1727, 1737–38, 1740–41 [167 L.Ed.2d 705], 82 U.S.P.Q.2d 1385, 1394, 1396 (2007) (citing *Deuel*, 51 F.3d at 1559). Under *KSR*, it's now apparent "obvious to try" may be an appropriate test in more situations than we previously contemplated.

Board Decision at 8. Insofar as *Deuel* implies the obviousness inquiry cannot consider that the combination of the claim's constituent elements was "obvious to try," the Supreme Court in *KSR* unambiguously discredited that holding. In fact, the Supreme Court expressly invoked *Deuel* as a source of the discredited "obvious to try" doctrine. The *KSR* Court reviewed this court's rejection, based on *Deuel*, of evidence showing that a particular combination of prior art elements was obvious because it would have been obvious to one of ordinary skill in the art to attempt such a combination:

> The only declaration offered by KSR—a declaration by its Vice President of Design Engineering, Larry Willemsen—did not go to the ultimate issue of motivation to combine prior art, i.e. whether one of ordinary skill in the art would have been motivated to attach an electronic control to the support bracket of the assembly disclosed by Asano. Mr. Willemsen did state that an electronic control "could have been" mounted on the support bracket of a pedal assembly.

(Willemsen Decl. at P33, 36, 39.) Such testimony is not sufficient to support a finding of obviousness, however. *See, e.g., In re Deuel*, 51 F.3d 1552, 1559 (Fed.Cir.1995) ("'Obvious to try' has long been held not to constitute obviousness.").

Teleflex, Inc. v. KSR Int'l Co., 119 Fed. Appx. 282, 289 (Fed.Cir.2005). The Supreme Court repudiated as "error" the *Deuel* restriction on the ability of a skilled artisan to combine elements within the scope of the prior art:

> The same constricted analysis led the Court of Appeals to conclude, in error, that a patent claim cannot be proved obvious merely by showing that the combination of elements was "obvious to try." When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance *the fact that a combination was obvious to try might show that it was obvious under § 103.*

KSR, 550 U.S. at 421, 127 S.Ct. 1727 (internal citation omitted) (emphasis added).

The Supreme Court's admonition against a formalistic approach to obviousness in this context actually resurrects this court's own wisdom in *In re O'Farrell*, which predates the *Deuel* decision by some seven years. This court in *O'Farrell* cautioned that "obvious to try" is an incantation whose meaning is often misunderstood:

> It is true that this court and its predecessors have repeatedly emphasized that "obvious to try" is not the standard under § 103. However, the meaning of this maxim is sometimes lost. Any invention that would in fact have been obvious under § 103 would also have been, in a sense, obvious to try. The question is: when is an invention that was obvious to try nevertheless nonobvious?

In re O'Farrell, 853 F.2d 894, 903 (Fed. Cir.1988). To differentiate between proper and improper applications of "obvious to try," this court outlined two classes of situations where "obvious to try" is erroneously equated with obviousness under § 103. In the first class of cases,

> what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.

Id. In such circumstances, where a defendant merely throws metaphorical darts at a board filled with combinatorial prior art possibilities, courts should not succumb to hindsight claims of obviousness. The inverse of this proposition is succinctly encapsulated by the Supreme Court's statement in *KSR* that where a skilled artisan merely pursues "known options" from a "finite number of identified, predictable solutions," obviousness under § 103 arises. 550 U.S. at 421, 127 S.Ct. 1727.

The second class of *O'Farrell*'s impermissible "obvious to try" situations occurs where

> what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

853 F.2d at 903. Again, *KSR* affirmed the logical inverse of this statement by stating

that § 103 bars patentability unless "the improvement is more than the predictable use of prior art elements according to their established functions." 550 U.S. at 417, 127 S.Ct. 1727.

This court in *O'Farrell* found the patentee's claims obvious because the Board's rejection of the patentee's claims had not presented either of the two common "obvious to try" pitfalls. Specifically, this court observed that an obviousness finding was appropriate where the prior art "contained *detailed enabling methodology* for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful." 853 F.2d at 902 (emphasis added). Responding to concerns about uncertainty in the prior art influencing the purported success of the claimed combination, this court stated: "[o]bviousness does not require absolute predictability of success ... *all that is required is a reasonable expectation of success.*" *Id.* at 903–04 (emphasis added). The Supreme Court in *KSR* reinvigorated this perceptive analysis.

KSR and *O'Farrell* directly implicate the instant case. Appellants' claim 73 recites a genus of isolated nucleic acid molecules encoding the NAIL protein. As found by the Board, the Valiante reference discloses the very protein of appellants' interest—"p38" as per Valiante. *Board Decision* at 4. Valiante discloses a monoclonal antibody mAb C1.7 that is specific for p38/NAIL, and further teaches a five-step protocol for cloning nucleic acid molecules encoding p38/NAIL using mAb C1.7. *Id.* In fact, while stating that "[t]he DNA and protein sequences for the receptor p38 may be obtained by resort to conventional methodologies known to one of skill in the art," '690 Patent at col.7 ll.49–51, Valiante cites to the *very same* cloning manual, Sambrook, cited by Kubin and Goodwin for their proposition that the gene sequence is identified and recovered "by standard biochemical methods." '859 Application at 16. Moreover, the record strongly reinforces (and appellants apparently find no room to dispute) the Board's factual finding that one of ordinary skill would have been motivated to isolate NAIL cDNA, given Valiante's teaching that p38 is "expressed by virtually all human NK cells and thus plays a role in the immune response." *Board Decision* at 6. The record shows that the prior art teaches a protein of interest, a motivation to isolate the gene coding for that protein, and illustrative instructions to use a monoclonal antibody specific to the protein for cloning this gene. Therefore, the claimed invention is "the product not of innovation but of ordinary skill and common sense." *KSR*, 550 U.S. at 421, 127 S.Ct. 1727. Or stated in the familiar terms of this court's long-standing case law, the record shows that a skilled artisan would have had a resoundingly "reasonable expectation of success" in deriving the claimed invention in light of the teachings of the prior art. *See O'Farrell*, 853 F.2d at 904.

This court also declines to cabin *KSR* to the "predictable arts" (as opposed to the "unpredictable art" of biotechnology). In fact, this record shows that one of skill in this advanced art would find these claimed "results" profoundly "predictable." The record shows the well-known and reliable nature of the cloning and sequencing techniques in the prior art, not to mention the readily knowable and obtainable structure of an identified protein. Therefore this court cannot deem irrelevant the ease and predictability of cloning the gene that codes for that protein. This court cannot, in the face of *KSR*, cling to formalistic rules for obviousness, customize its legal tests for specific scientific fields in ways that deem entire classes of prior art teachings irrelevant, or discount the significant abilities of artisans of ordinary skill in an advanced area of art. *See In re Durden,*

763 F.2d 1406, 1411 (Fed.Cir.1985) ("Our function is to apply, in each case, § 103 as written to the facts of disputed issues, not to generalize or make rules for other cases which are unforeseeable."). As this court's predecessor stated in *In re Papesch*, "[t]he problem of 'obviousness' under section 103 in determining the patentability of new and useful chemical compounds ... is not really a problem in chemistry or pharmacology or in any other related field of science such as biology, biochemistry, pharmacodynamics, ecology, or others yet to be conceived. It is a problem of patent law." 315 F.2d 381, 386 (CCPA 1963).

The record in this case shows that Valiante did not explicitly supply an amino acid sequence for NAIL or a polynucleotide sequence for the NAIL gene. In that sense, Kubin and Goodwin's disclosure represents some minor advance in the art. But "[g]ranting patent protection to advances that would occur in the ordinary course without real innovation retards progress." *KSR*, 550 U.S. at 419, 127 S.Ct. 1727. "Were it otherwise patents might stifle, rather than promote, the progress of useful arts." *Id.* at 427, 127 S.Ct. 1727. In light of the concrete, specific teachings of Sambrook and Valiante, artisans in this field, as found by the Board in its expertise, had every motivation to seek and every reasonable expectation of success in achieving the sequence of the claimed invention. In that sense, the claimed invention was reasonably expected in light of the prior art and "obvious to try." *See Ortho–McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364 (Fed.Cir.2008) ("KSR posits a situation with a finite, and in the context of the art, small or easily traversed, number of options that would convince an ordinarily skilled artisan of obviousness."). These references, which together teach a protein identical to NAIL, a commercially available monoclonal antibody specific for NAIL, and explicit instructions for obtaining the DNA sequence for NAIL, are not analogous to prior art that gives "no direction as to which of many possible choices is likely to be successful" or "only general guidance as to the particular form of the claimed invention or how to achieve it." *O'Farrell*, 853 F.2d at 903. As the Board found, the prior art here provides a "reasonable expectation of success" for obtaining a polynucleotide within the scope of claim 73, *Board Decision* at 6, which, "[f]or obviousness under § 103 [is] all that is required." *O'Farrell*, 853 F.2d at 903. Thus, this court affirms the Board's conclusion as to obviousness.

IV.

For the reasons stated above, the Board did not err in finding appellants' claims obvious as a matter of law. Thus, this court need not address appellants' contention that the Board erred in finding its claims invalid under § 112 ¶ 1. Accordingly, this court affirms the decision of the Board.

AFFIRMED.

COSTS

Each party shall bear its own costs.

PALMYRA PACIFIC SEAFOODS, L.L.C., Palmyra Pacific Enterprises, L.L.C., PPE Limited Partnership, and Frank Sorba, Plaintiffs–Appellants,

v.

UNITED STATES, Defendant–Appellee.

No. 2008–5058.

United States Court of Appeals, Federal Circuit.

April 9, 2009.

Background: Commercial fishing entities brought action against the United States

FEDERAL CASES

In re O'Farrell

sion with the admittedly famous name DEBEERS. No dispute is raised with respect to the alleged fact that the name DEBEERS has an established trade identity in the United States in connection with diamonds. We note particularly the factors of the fame of DEBEERS as a source for diamonds, the unusual nature of the name, and the identity of Ullenberg's goods with those associated with DBCM. The record also conclusively establishes that JVC and its members will be damaged if such confusion occurs.

Because JVC has established that registration of Ullenberg's alleged mark is precluded under section 2(d), we need not address the alternative section 2(a) grounds.[4]

V

For the foregoing reasons the board's decision dismissing JVC's opposition with prejudice for failure to state a claim upon which relief can be granted is reversed, and the case is remanded with directions that the board grant JVC's motion for summary judgment.

REVERSED AND REMANDED.

In re Patrick H. O'FARRELL, Barry A. Polisky and David H. Gelfand.

No. 87-1486.

United States Court of Appeals, Federal Circuit.

Aug. 10, 1988.

Appeal was taken from a decision of the Patent and Trademark Office Board of Patent Appeals and Interferences affirming patent examiner's final rejection of patent application entitled "Method and Hybrid Vector for Regulating Translation of Heterologous DNA in Bacteria." The Court of Appeals, Rich, Circuit Judge, held that patent would be rejected on grounds of obviousness.

Affirmed.

1. Patents ⇐314(5)

Obviousness of patent is a question of law. 35 U.S.C.A. § 103.

2. Patents ⇐16(1)

Analysis of obviousness of patent must be based on several factual inquiries: scope and content of prior art, differences between prior art and claims at issue, level of ordinary skill in the art at time invention was made, and objective evidence of nonobviousness, if any. 35 U.S.C.A. § 103.

3. Patents ⇐16.6

Patent application entitled "Method and Hybrid Vector for Regulating Translation of Heterologous DNA in Bacteria" was rejected on ground that claimed invention would have been obvious at time invention was made; application was obvious in view of published paper by two of three coinventors who, after providing virtually all of their method to public without applying for patent within a year, foreclosed themselves from obtaining patent on method that would have been obvious from their publication to those of ordinary skill in the art. 35 U.S.C.A. § 103.

4. Patents ⇐16(2)

For there to be obviousness of patent on basis of prior art, all that is required is reasonable expectation of success. 35 U.S.C.A. § 103.

J. Bruce McCubbrey, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., argued for appellant. Virginia H. Meyer, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., was on the brief for appellant.

Harris A. Pitlick, Associate Sol., of Arlington, Va., argued for appellee. With him on the brief were Joseph F. Nakamura, Sol. and Fred E. McKelvey, Deputy Sol.

4. All outstanding procedural motions filed in this appeal are denied.

Before MARKEY, Chief Judge, and RICH and NIES, Circuit Judges.

RICH, Circuit Judge.

This appeal is from the decision of the United States Patent and Trademark Office Board of Patent Appeals and Interferences (board) affirming the patent examiner's final rejection of patent application Serial No. 180,424, entitled "Method and Hybrid Vector for Regulating Translation of Heterologous DNA in Bacteria." The application was rejected under 35 U.S.C. § 103 on the ground that the claimed invention would have been obvious at the time the invention was made in view of a published paper by two of the three coinventors, and a publication by Bahl, Marians & Wu, 1 *Gene* 81 (1976) (Bahl). We affirm.

The claimed invention is from the developing new field of genetic engineering. A broad claim on appeal reads:

Claim 1. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria comprising, providing a cloning vector which includes at least a substantial portion of a gene which is indigenous to the host species of bacteria and is functionally transcribed and translated in that species, said substantial portion of said indigenous gene further including the regulatory DNA sequences for RNA synthesis and protein synthesis but lacking the normal gene termination signal, and linking a natural or synthetic heterologous gene encoding said predetermined protein to said indigenous gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of said indigenous gene portion so that readthrough can occur from said indigenous gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

Illustrative embodiments are defined in more specific claims. For example:

Claim 2. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria, comprising, providing an *E. coli* plasmid having an operator, a promoter, a site for the initiation of translation, and at least a substantial portion of the beta-galactosidase gene of the *E. coli* lactose operon, said substantial portion of said beta-galactosidase gene being under the control of said operator, promoter and site for initiation of translation, said substantial portion of said beta-galactosidase gene lacking the normal gene termination signal, and linking a heterologous gene encoding said predetermined protein to said beta-galactosidase gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of the said beta-galactosidase gene portion so that readthrough can occur from said beta-galactosidase gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

Claim 3. The method of Claim 2 wherein said *E. coli* plasmid comprises the plasmid designated pBGP120.

Although the terms in these claims would be familiar to those of ordinary skill in genetic engineering, they employ a bewildering vocabulary new to those who are not versed in molecular biology. An understanding of the science and technology on which these claims are based is essential before one can analyze and explain whether the claimed invention would have been obvious in light of the prior art.

I. *Background*[1]

Proteins are biological molecules of enormous importance. Proteins include en-

1. Basic background information about molecular biology and genetic engineering, can be

zymes that catalyze biochemical reactions, major structural materials of the animal body, and many hormones. Numerous patents and applications for patents in the field of biotechnology involve specific proteins or methods for making and using proteins. Many valuable proteins occur in nature only in minute quantities, or are difficult to purify from natural sources. Therefore, a goal of many biotechnology projects, including appellants' claimed invention, is to devise methods to synthesize useful quantities of specific proteins by controlling the mechanism by which living cells make proteins.

The basic organization of all proteins is the same. Proteins are large polymeric molecules consisting of chains of smaller building blocks, called *amino acids*, that are linked together covalently.[2] The chemical bonds linking amino acids together are called *peptide* bonds, so proteins are also called *polypeptides*.[3] It is the exact sequence in which the amino acids are strung together in a polypeptide chain that determines the identity of a protein and its chemical characteristics.[4] Although there are only 20 amino acids, they are strung together in different orders to produce the hundreds of thousands of proteins found in nature.

To make a protein molecule, a cell needs information about the sequence in which the amino acids must be assembled. The cell uses a long polymeric molecule, DNA (deoxyriboneucleic acid), to store this information. The subunits of the DNA chain are called *nucleotides*. A nucleotide consists of a nitrogen-containing ring compound (called a *base*) linked to a 5–carbon sugar that has a phosphate group attached.[5] DNA is composed of only four nucleotides. They differ from each other in the base region of the molecule. The four bases of these subunits are adenine, guanine, cytosine, and thymine (abbreviated respectively as A, G, C and T). The sequence of these bases along the DNA molecule specifies which amino acids will be inserted in sequence into the polypeptide chain of a protein.

DNA molecules do not participate directly in the synthesis of proteins. DNA acts as a permanent "blueprint" of all of the

found in Alberts, Bray, Lewis, Raff, Roberts & Watson, *The Molecular Biology of the Cell*, 1–253, 385–481 (1983) [hereinafter *The Cell*]; Watson, Hopkins, Roberts, Steitz & Weiner, *The Molecular Biology of the Gene*, Vol. 1 (4th ed., 1987) 3–502 [hereinafter *The Gene*]. These standard textbooks were used to supplement the information in the glossary supplied by appellants. The description here is necessarily simplified and omits important facts and concepts that are not necessary for the analysis of this case.

2. There are twenty amino acids: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

3. Proteins are often loosely called *peptides*, but technically proteins are only the larger peptides with chains of at least 50 amino acids, and more typically hundreds of amino acids. Some proteins consist of several polypeptide chains bound together covalently or noncovalently. The term "peptide" is broader than "protein" and also includes small chains of amino acids linked by peptide bonds, some as small as two amino acids. Certain small peptides have commercial or medical significance.

4. Polypeptide chains fold up into complex 3–dimensional shapes. It is the shape that actually determines many chemical properties of the protein. However, the configuration of a protein molecule is determined by its amino acid sequence. *The Cell* at 111–12; *The Gene* at 50–54.

5. The sugar in DNA is deoxyribose, while the sugar in RNA, *infra*, is ribose. The sugar and phosphate groups are linked covalently to those of adjacent nucleotides to form the backbone of the long unbranched DNA molecule. The bases project from the chain, and serve as the "alphabet" of the genetic code.

DNA molecules actually consist of two chains tightly entwined as a double helix. The chains are not identical but instead are complementary: each A on one chain is paired with a T on the other chain, and each C has a corresponding G. The chains are held together by noncovalent bonds between these complementary bases. This double helical structure plays an essential role in the replication of DNA and the transmission of genetic information. *See generally The Cell* at 98–106; *The Gene* at 65–79. However, the information of only one strand is used for directing protein synthesis, and it is not necessary to discuss the implication of the double-stranded structure of DNA here. RNA molecules, *infra*, are single stranded.

genetic information in the cell, and exists mainly in extremely long strands (called *chromosomes*) containing information coding for the sequences of many proteins, most of which are not being synthesized at any particular moment. The region of DNA on the chromosome that codes for the sequence of a single polypeptide is called a *gene.*[6] In order to *express* a gene (the process whereby the information in a gene is used to synthesize new protein), a copy of the gene is first made as a molecule of RNA (ribonucleic acid).

RNA is a molecule that closely resembles DNA. It differs, however, in that it contains a different sugar (ribose instead of deoxyribose) and the base thymine (T) of DNA is replaced in RNA by the structurally similar base, uracil (U). Making an RNA copy of DNA is called *transcription.* The transcribed RNA copy contains sequences of A, U, C, and G that carry the same information as the sequence of A, T, C, and G in the DNA. That RNA molecule, called *messenger RNA*, then moves to a location in the cell where proteins are synthesized.

The code whereby a sequence of nucleotides along an RNA molecule is translated into a sequence of amino acids in a protein (i.e., the "genetic code") is based on serially reading groups of three adjacent nucleotides. Each combination of three adjacent nucleotides, called a *codon*, specifies a particular amino acid. For example, the codon U–G–G in a messenger RNA molecule specifies that there will be a tryptophan molecule in the corresponding location in the corresponding polypeptide. The four bases A, G, C and U can be combined as triplets in 64 different ways, but there are only 20 amino acids to be coded. Thus, most amino acids are coded for by more than one codon. For example, both U–A–U and U–A–C code for tyrosine, and there are six different codons that code for leucine. There are also three codons that do not code for any amino acid (namely, U–A–A, U–G–A, and U–A–G). Like periods at the end of a sentence, these sequences signal the end of the polypeptide chain, and they are therefore called *stop codons.*

The cellular machinery involved in synthesizing proteins is quite complicated, and centers around large structures called *ribosomes* that bind to the messenger RNA. The ribosomes and associated molecules "read" the information in the messenger RNA molecule, literally shifting along the strand of RNA three nucleotides at a time, adding the amino acid specified by that codon to a growing polypeptide chain that is also attached to the ribosome. When a stop codon is reached, the polypeptide chain is complete and detaches from the ribosome.

The conversion of the information from a sequence of codons in an RNA molecule into the sequence of amino acids in a newly synthesized polypeptide is called *translation.* A messenger RNA molecule is typically reused to make many copies of the same protein. Synthesis of a protein is usually terminated by destroying the messenger RNA. (The information for making more of that protein remains stored in DNA in the chromosomes.)

The translation of messenger RNA begins at a specific sequence of nucleotides that bind the RNA to the ribosome and specify which is the first codon that is to be translated. Translation then proceeds by reading nucleotides, three at a time, until a stop codon is reached. If some error were to occur that shifts the frame in which the nucleotides are read by one or two nucleotides, all of the codons after this shift would be misread. For example, the sequence of codons [...*C–U–C–A–G–C–G–U–U–A–C–C–*A...] codes for the chain of amino acids [... leucine-serine-valine-threonine-...]. If the reading of these groups of three nucleotides is displaced by one nucleotide, such as [...*C–U–C–A–G–C–G–U–U–A–C–C–A*...], the resulting peptide chain would consist of

6. Chromosomes also contain regions of DNA that are not part of genes, i.e., do not code for the sequence of amino acids in proteins. These include sections of DNA adjacent to genes that are involved in the control of transcription, *infra*, and regions of unknown function.

[...serine-alanine-leucine-proline...]. This would be an entirely different peptide, and most probably an undesirable and useless one. Synthesis of a particular protein requires that the correct register or *reading frame* be maintained as the codons in the RNA are translated.

The function of messenger RNA is to carry genetic information (transcribed from DNA) to the protein synthetic machinery of a cell where its information is translated into the amino acid sequence of a protein. However, some kinds of RNA have other roles. For example, ribosomes contain several large strands of RNA that serve a structural function (*ribosomal RNA*). Chromosomes contain regions of DNA that code for the nucleotide sequences of structural RNAs and these sequences are transcribed to manufacture those RNAs. The DNA sequences coding for structural RNAs are still called genes even though the nucleotide sequence of the structural RNA is never translated into protein.

Man, other animals, plants, protozoa, and yeast are *eucaryotic* (or eukaryotic) organisms: their DNA is packaged in chromosomes in a special compartment of the cell, the nucleus. Bacteria (*procaryotic* or prokaryotic organisms) have a different organization. Their DNA, usually a circular loop, is not contained in any specialized compartment. Despite the incredible differences between them, all organisms, whether eucaryote or procaryote, whether man or mouse or lowly bacterium, use the same molecular rules to make proteins under the control of genes. In all organisms, codons in DNA are transcribed into codons in RNA which is translated on ribosomes into polypeptides according to the same genetic code. Thus, if a gene from a man is transferred into a bacterium, the bacterium can manufacture the human protein. Since most commercially valuable proteins come from man or other eucaryotes while bacteria are essentially little biochemical factories that can be grown in huge quantities, one strategy for manufacturing a desired protein (for example, insulin) is to transfer the gene coding for the protein from the eucaryotic cell where the gene normally occurs into a bacterium.

Bacteria containing genes from a foreign source (*heterologous* genes) integrated into their own genetic makeup are said to be *transformed*. When transformed bacteria grow and divide, the inserted heterologous genes, like all the other genes that are normally present in the bacterium (*indigenous* genes), are replicated and passed on to succeeding generations. One can produce large quantities of transformed bacteria that contain transplanted heterologous genes. The process of making large quantities of identical copies of a gene (or other fragment of DNA) by introducing it into procaryotic cells and then growing those cells is called *cloning* the gene. After growing sufficient quantities of the transformed bacteria, the biotechnologist must induce the transformed bacteria to *express* the cloned gene and make useful quantities of the protein. This is the purpose of the claimed invention.

In order to make a selected protein by expressing its cloned gene in bacteria, several technical hurdles must be overcome. First the gene coding for the specific protein must be isolated for cloning. This is a formidable task, but recombinant DNA technology has armed the genetic engineer with a variety of techniques to accomplish it.[7] Next the isolated gene must be introduced into the host bacterium. This can be done by incorporating the gene into a cloning vector. A *cloning vector* is a piece of DNA that can be introduced into bacteria and will then replicate itself as the bacterial cells grow and divide. Bacteriophage (viruses that infect bacteria) can be used as cloning vectors, but plasmids were the type used by appellants. A *plasmid* is a small circular loop of DNA found in bacteria, separate from the chromosome, that replicates like a chromosome. It is like a tiny auxilliary chromosome containing only a few genes. Because of their small size, plasmids are convenient for the molecular biologist to isolate and work with. Recombinant DNA technology can be used to modify plasmids by splicing in cloned eu-

7. See *The Cell* at 185–194; *The Gene* at 208–10.

caryotic genes and other useful segments of DNA containing control sequences. Short pieces of DNA can even be designed to have desired nucleotide sequences, synthesized chemically, and spliced into the plasmid. One use of such chemically synthesized linkers is to insure that the inserted gene has the same reading frame as the rest of the plasmid; this is a teaching of the Bahl reference cited against appellants. A plasmid constructed by the molecular geneticist can be inserted into bacteria, where it replicates as the bacteria grow.

Even after a cloned heterologous gene has been successfully inserted into bacteria using a plasmid as a cloning vector, and replicates as the bacteria grow, there is no guarantee that the gene will be expressed, i.e., transcribed and translated into protein. A bacterium such as *E. coli* (the species of bacterium used by appellants) has genes for several thousand proteins. At any given moment many of those genes are not expressed at all. The genetic engineer needs a method to "turn on" the cloned gene and force it to be expressed. This is the problem appellants worked to solve.

II. *Prior art*

Appellants sought to control the expression of cloned heterologous genes inserted into bacteria. They reported the results of their early efforts in a publication, the three authors of which included two of the three coinventor-appellants (the Polisky reference [8]), that is undisputed prior art against them. Their strategy was to link the foreign gene to a highly regulated indigenous gene. Turning on expression of the indigenous gene by normal control mechanisms of the host would cause expression of the linked heterologous gene.

As a controllable indigenous gene, the researchers chose a gene in the bacterium *E. coli* that makes beta-galactosidase. *Beta-galactosidase* is an enzyme needed to digest the sugar, lactose (milk sugar). When *E. coli* grows in a medium that contains no lactose, it does not make beta-galactosidase. If lactose is added to the medium, the gene coding for beta-galactosidase is expressed. The bacterial cell makes beta-galactosidase and is then able to use lactose as a food source. When lactose is no longer available, the cell again stops expressing the gene for beta galactosidase.

The molecular mechanisms through which the presence of lactose turns on expression of the beta-galactosidase gene has been studied in detail, and is one of the best understood examples of how gene expression is regulated on the molecular level. The beta-galactosidase gene is controlled by segments of DNA adjacent to the gene. These *regulatory DNA sequences* (the general term used in Claim 1) include the *operator* and *promoter* sequences (specified in Claim 2).[9] The researchers constructed a plasmid containing the beta-galactosidase gene with its operator and promoter. This gene (with its regulatory sequences) was removed from the chromosome of *E. coli* where it is normally found and was transplanted to a plasmid that could be conveniently manipulated.

Restriction endonucleases are useful tools in genetic engineering. These enzymes cut strands of DNA, but only at places where a specific sequence of nucleotides is present. For example, one restriction endonuclease, called *EcoRI*, cuts DNA only at sites where the nucleotide sequence is [...-G-A-A-T-T-C-...]. With restric-

8. Polisky, Bishop & Gelfand, *A plasmid cloning vehicle allowing regulated expression of eukaryotic DNA in bacteria*, 73 Proc.Nat'l Acad.Sci. USA 3900 (1976).

9. The *promoter* is a sequence of nucleotides where the enzyme that synthesizes RNA, *RNA polymerase*, attaches to the DNA to start the transcription of the beta-galactosidase gene. The *operator* is an overlapping DNA sequence that binds a small protein present in the cell, the lactose repressor protein. The lactose repressor protein binds to the operator and physically blocks the RNA polymerase from properly attaching to the promoter so that transcription cannot proceed. Lactose molecules interact with the lactose repressor protein and cause it to change its shape; after this change in shape it moves out of the way and no longer prevents the RNA polymerase from binding to the promoter. Messenger RNA coding for beta-galactosidase can then be transcribed. *See generally The Cell* at 438–39; *The Gene* at 474–80.

tion enzymes the genetic engineer can cut a strand of DNA at very specific sites into just a few pieces. With the help of "repair" enzymes, other pieces of DNA can be spliced onto the cut ends. The investigators found that the plasmid which they had constructed contained only two sequences that were cut by EcoRI. They were able to eliminate one of these sites that was unwanted. They were then left with a plasmid containing the beta-galactosidase gene with its regulatory sequences, and a single EcoRI site that was within the beta-galactosidase gene and close to its stop codon. They named this plasmid that they had constructed pBGP120.

The next step was to cut the plasmid open at its EcoRI site and insert a heterologous gene from another organism. The particular heterologous gene they chose to splice in was a segment of DNA from a frog that coded for ribosomal RNA. The frog gene was chosen as a test gene for reasons of convenience and availability. The new plasmid created by inserting the frog gene was similar to pBGP120, but its beta-galactosidase gene was incomplete. Some codons including the stop codon were missing from its end, which instead continued on with the sequence of the frog ribosomal RNA gene. The investigators named this new plasmid pBGP123. They inserted this plasmid back into *E. coli* and grew sufficient quantities for study. They then fed the *E. coli* with lactose. As they had intended, the lactose turned on transcription of the beta-galactosidase gene in the plasmid. RNA polymerase moved along the plasmid producing a strange new kind of RNA: Each long strand of RNA first contained codons for the messenger RNA for beta-galactosidase and then continued without interruption with the codons for the frog ribosomal RNA. Thus, there was *readthrough* transcription in which the RNA polymerase first transcribed the indigenous (beta-galactosidase) gene and then "read through," i.e., continued into and through the adjacent heterologous (frog ribosomal RNA) gene. Although the RNA produced was a hybrid, it nevertheless contained a nucleotide sequence dictated by DNA from a frog. The researchers had achieved the first controlled transcription of an animal gene inside a bacterium.

The researchers had used a gene coding for a ribosomal RNA as their heterologous test gene. Ribosomal RNA is not normally translated into protein. Nevertheless, they were obviously interested in using their approach to make heterologous proteins in bacteria. They therefore examined the beta-galactosidase made by their transformed bacteria. Patrick O'Farrell, who was not a coauthor of the Polisky paper but was to become a coinventor in the patent application, joined as a collaborator. They found that beta-galactosidase from the transformed bacteria had a higher molecular weight than was normal. They concluded that the bacteria must have used their strange new hybrid RNA like any other messenger RNA and translated it into protein. When the machinery of protein synthesis reached the premature end of the sequence coding for beta-galactosidase it continued right on, three nucleotides at a time, adding whatever amino acid was coded for by those nucleotides, until a triplet was reached with the sequence of a stop codon. The resulting polypeptide chains had more amino acids than normal beta-galactosidase, and thus a higher molecular weight. The researchers published their preliminary results in the Polisky article. They wrote:

> [I]f the normal translational stop signals for [beta]-galactosidase are missing in pBGP120, in-phase translational readthrough into adjacent inserted sequences might occur, resulting in a significant increase in the size of the [beta]-galactosidase polypeptide subunit. In fact, we have recently observed that induced cultures of pBGP123 contain elevated levels of [beta]-galactosidase of higher subunit molecular weight than wild-type enzyme (P. O'Farrell, unpublished experiments). We believe this increase results from translation of *Xenopus* [frog] RNA sequences covalently linked to [messenger] RNA for [beta]-galactosidase, resulting in a fused polypeptide.

Polisky at 3904.

Since ribosomal RNA is never translated in normal cells, the polypeptide chain pro-

duced by translating that chain was not a naturally occuring, identified protein. The authors of the Polisky paper explicitly pointed out that if one were to insert a heterologous gene coding for a protein into their plasmid, it should produce a "fused protein" consisting of a polypeptide made of beta-galactosidase plus the protein coded for by the inserted gene, joined by a peptide bond into a single continuous polypeptide chain:

> It would be interesting to examine the expression of a normally translated eukaryotic sequence in pBGP120. If an inserted sequence contains a ribosome binding site that can be utilized in bacteria, production of high levels of a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide. In the absence of an independent ribosome binding site, the eukaryotic sequence would be translated to yield a peptide covalently linked to [beta]-galactosidase. The extent of readthrough translation under *lac* control will depend on the number of translatable codons between the EcoRI site and the first in-phase nonsense [i.e., stop] codon in the inserted sequence.

Id.

III. *The Claimed Invention*

Referring back to Claims 1 through 3, it can be seen that virtually everything in the claims was present in the prior art Polisky article. The main difference is that in Polisky the heterologous gene was a gene for ribosomal RNA while the claimed invention substitutes a gene coding for a predetermined protein. Ribosomal RNA gene is not normally translated into protein, so expression of the heterolgous gene was studied mainly in terms of transcription into RNA. Nevertheless, Polisky mentioned preliminary evidence that the transcript of the ribosomal RNA gene was translated into protein. Polisky further predicted that if a gene that codes for a protein were to be substituted for the ribosomal RNA gene, "a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide." Thus, the prior art explicitly suggested the substitution that is the difference between the claimed invention and the prior art, and presented preliminary evidence suggesting that the method could be used to make proteins.

Appellants reduced their invention to practice some time in 1976 and reported their results in a paper that was published in 1978.[10] During 1977 they communicated their results to another group of researchers who used the readthrough translation approach to achieve the first synthesis of a human protein in bacteria.[11] Appellants filed an application to patent their invention on August 9, 1978, of which the application on appeal is a division.

IV. *The Obviousness Rejection*

The application was rejected under 35 U.S.C. § 103. The position of the examiner and the Board is, simply, that so much of the appellant's method was revealed in the Polisky reference that making a protein by substituting its gene for the ribosomal RNA gene in Polisky (as suggested by Polisky) would have been obvious to one of ordinary skill in the art at the time that the invention was made.

The claims specify that the heterologous gene should be inserted into the plasmid in the same orientation and with the same reading frame as the preceding portion of

10. O'Farrell, Polisky & Gelfand, *Regulated expression by readthrough translation from a plasmid-encoded beta-galactosidase,* 134 J. Bacteriol. 645 (1978). The heterologous genes expressed in these studies were not predetermined, but were instead unidentified genes of unknown origin. The authors speculated that they were probably genes from *E. coli* that were conlaminants in the source of beta-galaclosidase genes. *Id.* at 648.

11. Itakura, Hirose, Crea, Riggs, Heynecker, Bolivar & Boyer, *Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin,* 198 Science 1056 (1977). A pioneering accomplishment of the Itakura group is that the gene was not from a human source, but instead was entirely synthesized in the laboratory using chemical methods. It is not clear whether the appellants communicated only the results reported in the Polisky publication or whether they communicated the complete claimed invention.

the indigenous gene. In view of this limitation, the § 103 rejection was based either on Polisky alone (supplemented by the fact that the importance of orientation and reading frame was well known in the prior art) or in combination with the Bahl reference which describes a general method for inserting a piece of chemically synthesized DNA into a plasmid. Bahl teaches that this technique could be used to shift the sequence of DNA inserted into a plasmid into the proper reading frame.

Appellants argue that at the time the Polisky article was published, there was significant unpredictability in the field of molecular biology so that the Polisky article would not have rendered the claimed method obvious to one of ordinary skill in the art. Even though there was speculation in the article that genes coding for proteins could be substituted for the ribosomal RNA gene and would be expressed as readthrough translation into the protein, this had never been done. Appellants say that it was not yet certain whether a heterologous protein could actually be produced in bacteria, and if it could, whether additional mechanisms or methods would be required. They contend that without such certainty the predictions in the Polisky paper, which hindsight now shows to have been correct, were merely invitations to those skilled in the art to try to make the claimed invention. They argue that the rejection amounts to the application of a standard of "obvious to try" to the field of molecular biology, a standard which this court and its predecessors have repeatedly rejected as improper grounds for a § 103 rejection. *E.g., In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed.Cir.1988); *In re Geiger*, 815 F.2d 686, 688, 2 USPQ2d 1276, 1278 (Fed.Cir.1987); *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097, 231 USPQ 375, 379 (Fed.Cir.1986); *In re Antonie*, 559 F.2d 618, 620, 195 USPQ 6, 8 (CCPA 1977).

[1, 2] Obviousness under § 103 is a question of law. *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561, 1568, 1 USPQ2d 1593, 1597 (Fed.Cir.), *cert. denied*, — U.S. —, 107 S.Ct. 2187, 95 L.Ed.2d 843 (1987). An analysis of obviousness must be based on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art at the time the invention was made; and (4) objective evidence of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18, 86 S.Ct. 684, 693–94, 15 L.Ed.2d 545, 556–57, 148 USPQ 459, 467 (1966). *See, e.g., Custom Accessories, Inc. v. Jeffrey-Allan Indus.*, 807 F.2d 955, 958, 1 USPQ2d 1196, 1197 (Fed.Cir.1986). The scope and content of the prior art and the differences between the prior art and the claimed invention have been examined in sections II and III, *supra*. Appellants say that in 1976 those of ordinary skill in the arts of molecular biology and recombinant DNA technology were research scientists who had "extraordinary skill in relevant arts" and "were among the brightest biologists in the world." Objective evidence of nonobviousness was not argued.

[3] With the statutory factors as expounded by *Graham* in mind and considering all of the evidence, this court must determine the correctness of the board's legal determination that the claimed invention as a whole would have been obvious to a person having ordinary skill in the art at the time the invention was made. We agree with the board that appellants' claimed invention would have been obvious in light of the Polisky reference alone or in combination with Bahl within the meaning of § 103. Polisky contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful.

Appellants argue that after the publication of Polisky, successful synthesis of protein was still uncertain. They belittle the predictive value of the observation that expression of the transcribed RNA in Polisky produced beta-galactosidase with a greater than normal molecular weight, arguing that since ribosomal RNA is not normally translated, the polypeptide chains that were added to the end of the beta-galactosi-

dase were "junk" or "nonsense" proteins. This characterization ignores the clear implications of the reported observations. The Polisky study directly proved that a readthrough transcript messenger RNA had been produced. The preliminary observation showed that this messenger RNA was read and used for successful translation. It was well known in the art that ribosomal RNA was made of the same nucleotides as messenger RNA, that any sequence of nucleotides could be read in groups of three as codons, and that reading these codons should specify a polypeptide chain that would elongate until a stop codon was encountered. The preliminary observations thus showed that codons beyond the end of the beta-galactosidase gene were being translated into peptide chains. This would reasonably suggest to one skilled in the art that if the codons inserted beyond the end of the beta-galactosidase gene coded for a "predetermined protein," that protein would be produced. In other words, it would have been obvious and reasonable to conclude from the observation reported in Polisky that since nonsense RNA produced nonsense polypeptides, if meaningful RNA was inserted instead of ribosomal RNA, useful protein would be the result. The relative shortness of the added chains is also not a source of uncertainty, since one skilled in the art would have known that a random sequence of nucleotides would produce a stop codon before the chain got too long.[12]

Appellants complain that since predetermined proteins had not yet been produced in transformed bacteria, there was uncertainty as to whether this could be done, and that the rejection is thus founded on an impermissible "obvious to try" standard. It is true that this court and its predecessors have repeatedly emphasized that "obvious to try" is not the standard under § 103. However, the meaning of this maxim is sometimes lost. Any invention that would in fact have been obvious under § 103 would also have been, in a sense, obvious to try. The question is: when is an invention that was obvious to try nevertheless nonobvious?

The admonition that "obvious to try" is not the standard under § 103 has been directed mainly at two kinds of error. In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. *E.g., In re Geiger*, 815 F.2d at 688, 2 USPQ2d at 1278; *Novo Industri A/S v. Travenol Laboratories, Inc.*, 677 F.2d 1202, 1208, 215 USPQ 412, 417 (7th Cir.1982); *In re Yates*, 663 F.2d 1054, 1057, 211 USPQ 1149, 1151 (CCPA 1981); *In re Antonie*, 559 F.2d at 621, 195 USPQ at 8–9. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed.Cir.1988); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 90–91 (Fed.Cir.1986), *cert. denied*, — U.S. ——, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987); *In re Tomlinson*, 363 F.2d 928, 931, 150 USPQ 623, 626 (CCPA 1966). Neither of these situations applies here.

[4] Obviousness does not require absolute predictability of success. Indeed, for many inventions that seem quite obvious, there is no absolute predictability of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious. *In re Merck & Co.*, 800 F.2d at 1098, 231 USPQ at 380; *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d

12. The patent application indicates that chains as long as 60 amino acids were added, which is hardly a trivial length of polypeptide.

1452, 1461, 221 USPQ 481, 488 (Fed.Cir. 1984); *In re Papesch*, 315 F.2d 381, 386–87, 137 USPQ 43, 47–48 (CCPA 1963). For obviousness under § 103, all that is required is a reasonable expectation of success. *In re Longi*, 759 F.2d 887, 897, 225 USPQ 645, 651–52 (Fed.Cir.1985); *In re Clinton*, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976). The information in the Polisky reference, when combined with the Bahl reference provided such a reasonable expectation of success.

Appellants published their pioneering studies of the expression of frog ribosomal RNA genes in bacteria more than a year before they applied for a patent. After providing virtually all of their method to the public without applying for a patent within a year, they foreclosed themselves from obtaining a patent on a method that would have been obvious from their publication to those of ordinary skill in the art, with or without the disclosures of other prior art. The decision of the board is

AFFIRMED.

SPERRY CORPORATION and Sperry World Trade, Inc., Plaintiffs–Appellants,

v.

The UNITED STATES, Defendant–Appellee.

No. 88–1009.

United States Court of Appeals, Federal Circuit.

Aug. 10, 1988.

American corporation which had obtained award from Iran-United States Claims Tribunal brought action challenging federal Government's deduction of expenses. The United States Claims Court, Loren A. Smith, Chief Judge, 12 Cl.Ct. 736, held that statute authorizing deduction was constitutional, and corporation appealed. The Court of Appeals, Mayer, Circuit Judge, held that statute authorizing deduction from Iran-United States Claims Tribunal awards, to reimburse federal Government for costs of arbitration, was per se taking of property, which was without compensation, and thus unconstitutional.

Reversed and remanded.

Eminent Domain ⇐2(1.1)

Statute authorizing deduction from Iran-United States Claims Tribunal awards, to reimburse federal Government for costs of arbitration, was per se taking of property, which was without compensation, and thus unconstitutional; creditor had sufficient forum and remedy in court, where it would have suffered no deduction from its judgment, until statute suspended its court claim in favor of imposed arbitration. U.S. C.A. Const.Amend. 5; Foreign Relations Authorization Act, Fiscal Years 1986 and 1987, § 502, 50 U.S.C.A. § 1701 note.

John D. Seiver, Cole, Raywid & Braverman, Washington, D.C., argued for plaintiffs-appellants. With him on the brief were Alan Raywid and Susan Paradise Baxter, Washington, D.C.

Terrence S. Hartman, Commercial Litigation Branch, Dept. of Justice, Washington, D.C., argued for defendant-appellee. With him on the brief were James M. Spears, Acting Asst. Atty. Gen. and David M. Cohen, Director, Washington, D.C. Also on the brief were Russell Munk and Francine Barber, Dept. of the Treasury and Ronald Bettauer and Lisa Grosh, Dept. of State, Washington, D.C., of counsel.

Before MARKEY, Chief Judge, COWEN, Senior Circuit Judge, and MAYER, Circuit Judge.

OPINION

MAYER, Circuit Judge.

Sperry Corporation and Sperry World Trade, Inc. (Sperry) appeal the judgment of

FEDERAL CASES

Takeda Chemical Industries v. Alphapharm Pty.

Takeda Chemical Industries v. Alphapharm Pty.

ence was before the examiner, whether through the examiner's search or the applicant's disclosure, it can not be deemed to have been withheld from the examiner.").

Because the district court clearly erred in determining that the statements in the October 2005 Response were affirmative misrepresentations of material fact and because there was no failure to disclose information while the reexamination was still pending before the PTO, we conclude that the district court erred in granting summary judgment of inequitable conduct.

CONCLUSION

Because we conclude that the term "near" is not indefinite, we reverse the district court's grant of judgment of invalidity for indefiniteness. Because we determine that no affirmative misrepresentation of material fact occurred and that there was not a failure to timely disclose material information, we reverse the summary judgment of unenforceability.

REVERSED

TAKEDA CHEMICAL INDUSTRIES, LTD. and Takeda Pharmaceuticals North America, INC., Plaintiffs–Appellees,

v.

ALPHAPHARM PTY., LTD. and Genpharm, Inc., Defendants–Appellants.

No. 06–1329.

United States Court of Appeals, Federal Circuit.

June 28, 2007.

Background: Owner of patent for diabetes drug brought infringement actions against proposed manufacturers of generic versions. The United States District Court for the Southern District of New York, Denise Cote, J., 417 F.Supp.2d 341, granted judgment for owner. Manufacturers appealed.

Holdings: The Court of Appeals, Lourie, Circuit Judge, held that:

(1) person of ordinary skill in the art would not have selected closest prior art compound as lead compound for antidiabetic treatment;

(2) person of ordinary skill in the art would not have been prompted to modify closest prior art compound, using steps of homologation or ring-walking, to synthesize claimed compound; and

(3) any error was harmless that district court may have committed by incorrectly implying that prosecution histories were not accessible to public.

Affirmed.

Dyk, Circuit Judge, filed concurring opinion.

1. Patents ⇐16.25

Person of ordinary skill in the art would not have selected closest prior art compound as lead compound for antidiabetic treatment, and thus presumption of motivation did not apply on competitor's claim of obviousness; although prosecution history of patent included statement characterizing compound as "especially important," any suggestion to select compound was essentially negated given more exhaustive and reliable scientific analysis which taught away from compound and evidence from similar contemporaneously filed patents showed that there were many promising, broad avenues for further research. 35 U.S.C.A. § 103.

2. Patents ⚖312(4)

Because a patent is presumed to be valid, the evidentiary burden to show facts supporting a conclusion of invalidity, which rests on the accused infringer, is one of clear and convincing evidence. 35 U.S.C.A. § 282.

3. Patents ⚖324.5, 324.55(4)

Whether an invention would have been obvious is a question of law, reviewed de novo, based upon underlying factual questions which are reviewed for clear error following a bench trial. 35 U.S.C.A. § 103.

4. Patents ⚖16(2, 3), 36.1(1)

The factors that control an obviousness inquiry are: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims; (3) the level of ordinary skill in the pertinent art; and (4) objective evidence of nonobviousness. 35 U.S.C.A. § 103.

5. Patents ⚖16.25

In a case involving a patent on a new chemical compound, some reason must be identified that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound. 35 U.S.C.A. § 103.

6. Patents ⚖16.25

Person of ordinary skill in the art would not have been prompted to modify closest prior art compound, using steps of homologation or ring-walking, to synthesize claimed compound in patent for antidiabetic treatment, and thus claimed compound was not obvious, where process of modifying lead compounds was not routine at time of invention, nothing in prior art provided reasonable expectation that adding methyl group to compound would have reduced or eliminated toxicity of lead compound, there was no reasonable expectation in the art that changing positions of substituent on pyridyl ring would have resulted in beneficial changes, and claimed compound differed significantly from lead compound, of which it was not a homolog, in terms of toxicity. 35 U.S.C.A. § 103.

7. Patents ⚖168(2.1)

Statement made during prosecution of patent for antidiabetic treatment in response to enablement rejection, indicating only that changes to left moiety of lead compound would create compounds with same properties as compounds of prior art, did not represent that lower toxicity would result from change, for purpose of obviousness claim. 35 U.S.C.A. § 103.

8. Patents ⚖324.56

Any error was harmless that district court may have committed by incorrectly implying that prosecution histories were not accessible to public, on competitor's claim of obviousness, where court nonetheless considered prosecution history of patent in its obviousness analysis and accorded proper weight to statements contained therein. 35 U.S.C.A. § 103.

Patents ⚖328(2)

4,287,200. Cited as Prior Art.

Patents ⚖328(2)

4,340,605, 4,438,141, 4,444,779. Cited.

Patents ⚖328(2)

4,687,777. Valid.

David G. Conlin, Edwards Angell Palmer & Dodge LLP, of Boston, MA, argued

for plaintiffs-appellees. With him on the brief were Barbara L. Moore, Kathleen B. Carr, and Adam P. Samansky; and Anthony J. Viola and Andre K. Cizmarik, of New York, NY. Of counsel on the brief was Mark Chao, Takeda Pharmaceuticals North America, Inc., of Lincolnshire, IL.

Kevin F. Murphy, Frommer Lawrence & Haug LLP, of New York, NY, argued for defendants-appellants. With him on the brief were Edgar H. Haug and Jeffrey A. Hovden.

Before LOURIE, BRYSON, and DYK, Circuit Judges.

Opinion for the court filed by Circuit Judge LOURIE. Concurring opinion filed by Circuit Judge DYK.

LOURIE, Circuit Judge.

Alphapharm Pty., Ltd. and Genpharm, Inc. (collectively "Alphapharm") appeal from the decision of the United States District Court for the Southern District of New York, following a bench trial, that U.S. Patent 4,687,777 was not shown to be invalid under 35 U.S.C. § 103. *Takeda Chem. Indus., Ltd. v. Mylan Labs.*, 417 F.Supp.2d 341 (S.D.N.Y.2006). Because we conclude that the district court did not err in determining that the claimed compounds would not have been obvious in light of the prior art, and hence that the patent has not been shown to be invalid, we affirm.

BACKGROUND

Diabetes is a disease that is characterized by the body's inability to regulate blood sugar. It is generally caused by inadequate levels of insulin—a hormone produced in the pancreas. Insulin allows blood sugar or glucose, which is derived from food, to enter into the body's cells and be converted into energy. There are two types of diabetes, known as Type 1 and Type 2. In Type 1 diabetes, the pancreas fails to produce insulin, and individuals suffering from this type of diabetes must regularly receive insulin from an external source. In contrast, Type 2 diabetic individuals produce insulin. However, their bodies are unable to effectively use the insulin that is produced. This is also referred to as insulin resistance. As a result, glucose is unable to enter the cells, thereby depriving the body of its main source of energy. Type 2 diabetes is the most common form of diabetes—affecting over 90% of diabetic individuals.

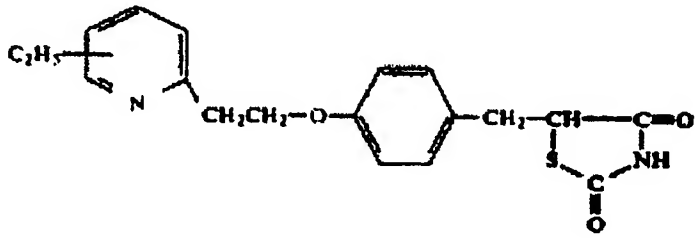
In the 1990s, a class of drugs known as thiazolidinediones ("TZDs") was introduced on the market as a treatment for Type 2 diabetes. Takeda Chemical Industries, Ltd., and Takeda Pharmaceuticals North America, Inc. (collectively "Takeda") first invented certain TZDs in the 1970s. Takeda's research revealed that TZDs acted as insulin sensitizers, *i.e.*, compounds that ameliorate insulin resistance. Although the function of TZDs was not completely understood, TZDs appeared to lower blood glucose levels by binding to a molecule in the nucleus of the cell known as PPARgamma, which activates insulin receptors and stimulates the production of glucose transporters. *Takeda*, 417 F.Supp.2d at 348–49. The transporters then travel to the cellular surface and enable glucose to enter the cell from the bloodstream. *Id.*

Takeda developed the drug ACTOS®, which is used to control blood sugar in patients who suffer from Type 2 diabetes. ACTOS® has enjoyed substantial commercial success since its launch in 1999. By

2003, it held 47% of the TZD market, and gross sales for that year exceeded $1.7 billion. *Id.* at 386. The active ingredient in ACTOS® is the TZD compound pioglitazone, a compound claimed in the patent in suit.

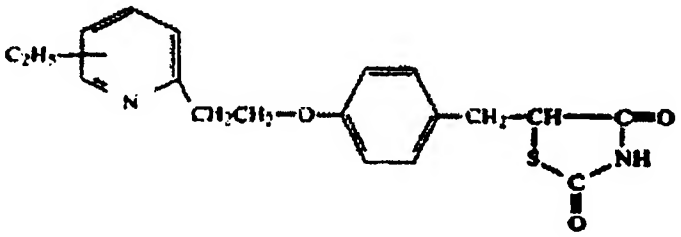
Takeda owns U.S. Patent 4,687,777 (the "'777 patent") entitled "Thiazolidinedione Derivatives, Useful As Antidiabetic Agents." The patent is directed to "compounds which can be practically used as antidiabetic agents having a broad safety margin between pharmacological effect and toxicity or unfavorable side reactions." '777 patent col.1 ll.34–37. The asserted claims are claims 1, 2, and 5. Claim 1 claims a genus of compounds. Claim 5 claims pharmaceutical compositions containing that genus of compounds. Those claims read as follows:

1. A compound of the formula:



or a pharmacologically acceptable salt thereof.

5. An antidiabetic composition which consists essentially of a compound of the formula:



or a pharmacologically acceptable salt thereof, in association with a pharmacologically acceptable carrier, excipient or diluent.

Id., claims 1 & 5.

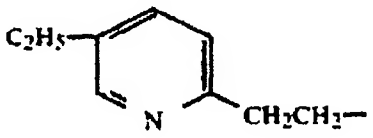
For purposes of this appeal, the critical portion of the compound structure is the left moiety of the molecule, namely, the ethyl-substituted pyridyl ring.[1] That chemical structure, which has an ethyl substituent (C^2 H^5) pictorially drawn to the center of the pyridyl ring, indicates that the structure covers four possible compounds, *viz.*, compounds with an ethyl substituent located at the four available positions on the pyridyl ring. *Takeda,* 417 F.Supp.2d at 360. The formula includes the 3–ethyl compound, 4–ethyl compound, 5–ethyl compound (pioglitazone), and 6–ethyl compound.

1. Pyridine is a "six-membered carbon-containing ring with one carbon replaced by a nitrogen." *Takeda,* 417 F.Supp.2d at 351.

Claim 2 of the '777 patent covers the single compound pioglitazone. That claim, which depends from claim 1, reads:

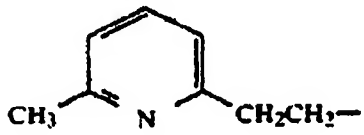
> 2. A compound as claimed in claim 1, wherein the compound is 5–{4–[2–(5–ethyl–2–pyridyl)ethoxy] benzyl}–2,4–thiazolidinedione.

'777 patent, claim 2. Pioglitazone is referred to as the 5–ethyl compound because the ethyl substituent is attached to the 5–position on the pyridyl ring. That portion of the compound is depicted as:



Alphapharm, a generic drug manufacturer, filed an Abbreviated New Drug Application ("ANDA") pursuant to the Hatch–Waxman Act seeking U.S. Food and Drug Administration ("FDA") approval under 21 U.S.C. § 355(j) et seq. to manufacture and sell a generic version of pioglitazone. Alphapharm filed a Paragraph IV certification with its ANDA pursuant to § 505(j)(2)(B)(ii), asserting that the '777 patent is invalid as obvious under 35 U.S.C. § 103. In response, Takeda sued Alphapharm, along with three other generic drug manufacturers who also sought FDA approval to market generic pioglitazone, alleging that the defendants have infringed or will infringe the '777 patent.

On January 17, 2006, the district court commenced a bench trial solely on the issues of validity and enforceability of the '777 patent. Alphapharm advanced its invalidity argument, asserting that the claimed compounds would have been obvious at the time of the alleged invention. Alphapharm's obviousness contention rested entirely on a prior art TZD compound that is referenced in Table 1 of the '777 patent as compound b. The left moiety of compound b consists of a pyridyl ring with a methyl (CH^3) group attached to the 6–position of the ring. That portion of its chemical structure is illustrated as follows:



Alphapharm asserted that the claimed compounds would have been obvious over compound b.

The district court found that Alphapharm failed to prove by clear and convincing evidence that the asserted claims were invalid as obvious under 35 U.S.C. § 103. The court first concluded that there was no motivation in the prior art to select compound b as the lead compound for antidiabetic research, and that the prior art taught away from its use. As such, the court concluded that Alphapharm failed to make a prima facie case of obviousness. The court continued its analysis and found that even if Alphapharm succeeded in making a prima facie showing, Takeda would still prevail because any prima facie case of obviousness was rebutted by the unexpected results of pioglitazone's nontoxicity. The court then rendered judgment in favor of Takeda. The district court also held that the '777 patent had not been procured though inequitable conduct. That decision has been separately appealed and has been affirmed in a decision issued today.

Alphapharm timely appealed. We have jurisdiction pursuant to 28 U.S.C. § 1295(a)(1).

DISCUSSION

A. *Standard of Review*

[1–3] In this appeal, we are presented with one issue, namely, whether the as-

serted claims of the '777 patent would have been obvious under 35 U.S.C. § 103 at the time the invention was made. An invention is not patentable, *inter alia*, "if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103(a). Because a patent is presumed to be valid, 35 U.S.C. § 282, the evidentiary burden to show facts supporting a conclusion of invalidity, which rests on the accused infringer, is one of clear and convincing evidence. *AK Steel Corp. v. Sollac & Ugine*, 344 F.3d 1234, 1238–39 (Fed.Cir. 2003). Whether an invention would have been obvious under 35 U.S.C. § 103 is a "question of law, reviewed de novo, based upon underlying factual questions which are reviewed for clear error following a bench trial." *Alza Corp. v. Mylan Labs., Inc.*, 464 F.3d 1286, 1289 (Fed.Cir.2006).

B. Obviousness

Alphapharm raises three main arguments in support of its contention that the claims would have been obvious. First, Alphapharm asserts that the district court misapplied the law, particularly the law governing obviousness in the context of structurally similar chemical compounds. According to Alphapharm, the record established that compound b was the most effective antidiabetic compound in the prior art, and thus the court erred by failing to apply a presumption that one of ordinary skill in the art would have been motivated to make the claimed compounds. Alphapharm asserts that such a conclusion is mandated by our case law, including our en banc decision in *In re Dillon*, 919 F.2d 688 (Fed.Cir.1990). Second, Alphapharm argues that the court erred in determining the scope and content of the prior art, in particular, whether to include the prosecution history of the prior '779 patent. Lastly, Alphapharm assigns error to numerous legal and factual determinations and certain evidentiary rulings that the court made during the course of the trial.

Takeda responds that the district court correctly determined that Alphapharm failed to prove by clear and convincing evidence that the asserted claims are invalid as obvious. Takeda contends that there was overwhelming evidence presented at trial to support the court's conclusion that no motivation existed in the prior art for one of ordinary skill in the art to select compound b as a lead compound, and even if there was, that the unexpected results of pioglitazone's improved toxicity would have rebutted any prima facie showing of obviousness. Takeda further argues that all of Alphapharm's remaining challenges to the district court's legal and factual rulings are simply without merit.

[4] We agree with Takeda that the district court did not err in concluding that the asserted claims of the '777 patent would not have been obvious. The Supreme Court recently addressed the issue of obviousness in *KSR International Co. v. Teleflex Inc.*, — U.S. —, 127 S.Ct. 1727, 167 L.Ed.2d 705 (2007). The Court stated that the *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 86 S.Ct. 684, 15 L.Ed.2d 545 (1966), factors still control an obviousness inquiry. Those factors are: 1) "the scope and content of the prior art"; 2) the "differences between the prior art and the claims"; 3) "the level of ordinary skill in the pertinent art"; and 4) objective evidence of nonobviousness. *KSR*, 127 S.Ct. at 1734 (quoting *Graham*, 383 U.S. at 17–18, 86 S.Ct. 684).

In a thorough and well-reasoned opinion, albeit rendered before *KSR* was decided

by the Supreme Court, the district court made extensive findings of fact and conclusions of law as to the four *Graham* factors. Alphapharm's arguments challenge the court's determinations with respect to certain of these factors, which we now address.

1. Differences Between the Prior Art and the Claims

a. Selection of Compound b as Lead Compound

Alphapharm's first argument challenges the court's determination with regard to the "differences between the prior art and the claims." Alphapharm contends that the court erred as a matter of law in holding that the ethyl-substituted TZDs were nonobvious in light of the closest prior art compound, compound b, by misapplying the law relating to obviousness of chemical compounds.

We disagree. Our case law concerning prima facie obviousness of structurally similar compounds is well-established. We have held that "structural similarity between claimed and prior art subject matter, proved by combining references or otherwise, where the prior art gives reason or motivation to make the claimed compositions, creates a prima facie case of obviousness." *Dillon*, 919 F.2d at 692. In addition to structural similarity between the compounds, a prima facie case of obviousness also requires a showing of "adequate support in the prior art" for the change in structure. *In re Grabiak*, 769 F.2d 729, 731–32 (Fed.Cir.1985).

We elaborated on this requirement in the case of *In re Deuel*, 51 F.3d 1552, 1558 (Fed.Cir.1995), where we stated that "[n]ormally a prima facie case of obviousness is based upon structural similarity, *i.e.*, an established structural relationship between a prior art compound and the claimed compound." That is so because close or established "[s]tructural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds." *Id.* A known compound may suggest its homolog, analog, or isomer because such compounds "often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties." *Id.* We clarified, however, that in order to find a prima facie case of unpatentability in such instances, a showing that the "prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention" was also required. *Id.* (citing *In re Jones*, 958 F.2d 347 (Fed.Cir.1992); *Dillon*, 919 F.2d 688; *Grabiak*, 769 F.2d 729; *In re Lalu*, 747 F.2d 703 (Fed.Cir.1984)).

[5] That test for prima facie obviousness for chemical compounds is consistent with the legal principles enunciated in *KSR*.[2] While the *KSR* Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to com-

2. We note that the Supreme Court in its *KSR* opinion referred to the issue as whether claimed subject matter "was" or "was not" obvious. Since 35 U.S.C. § 103 uses the language "would have been obvious," and the Supreme Court in *KSR* did consider the particular time at which obviousness is determined, we consider that the Court did not in *KSR* reject the standard statutory formulation of the inquiry whether the claimed subject matter "would have been obvious at the time the invention was made." 35 U.S.C. § 103. Hence, we will continue to use the statutory "would have been" language.

bine the elements in the way the claimed new invention does" in an obviousness determination. *KSR*, 127 S.Ct. at 1731. Moreover, the Court indicated that there is "no necessary inconsistency between the idea underlying the TSM test and the *Graham* analysis." *Id.* As long as the test is not applied as a "rigid and mandatory" formula, that test can provide "helpful insight" to an obviousness inquiry. *Id.* Thus, in cases involving new chemical compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound.

We agree with Takeda and the district court that Alphapharm failed to make that showing here. Alphapharm argues that the prior art would have led one of ordinary skill in the art to select compound b as a lead compound. By "lead compound," we understand Alphapharm to refer to a compound in the prior art that would be most promising to modify in order to improve upon its antidiabetic activity and obtain a compound with better activity.[3] Upon selecting that compound for antidiabetic research, Alphapharm asserts that one of ordinary skill in the art would have made two obvious chemical changes: first, homologation, *i.e.*, replacing the methyl group with an ethyl group, which would have resulted in a 6–ethyl compound; and second, "ring-walking," or moving the ethyl substituent to another position on the ring, the 5–position, thereby leading to the discovery of pioglitazone. Thus, Alphapharm's obviousness argument clearly depends on a preliminary finding that one of ordinary skill in the art would have selected compound b as a lead compound.

The district court found, however, that one of ordinary skill in the art would not have selected compound b as the lead compound. In reaching its determination, the court first considered Takeda's U.S. Patent 4,287,200 (the "'200 patent"), which was issued on September 1, 1981, and its prosecution history. The court found that the '200 patent "discloses hundreds of millions of TZD compounds."[4] *Takeda*, 417 F.Supp.2d at 378. The patent specifically identified fifty-four compounds, including compound b, that were synthesized according to the procedures described in the patent, but did not disclose experimental data or test results for any of those compounds. The prosecution history, however, disclosed test results for nine specific compounds, including compound b. That information was provided to the examiner in response to a rejection in order to show that the claimed compounds of the '200 patent were superior to the known compounds that were disclosed in a cited reference. The court, however, found nothing in the '200 patent, or in its file history, to suggest to one of ordinary skill in the art that those nine compounds, out of the hundreds of millions of compounds covered by the patent application, were the best performing compounds as antidiabetics, and hence targets for modification to seek improved properties. *Id.* at 375.

3. The parties do not dispute that compound b was the closest prior art compound. Thus, the legal question is whether or not the claimed subject matter would have been obvious over that compound. We will, however, use Alphapharm's terminology of "lead compound" in this opinion, deciding the appeal as it has been argued.

4. Three divisional applications derive from the '200 patent. Those applications matured into U.S. Patent 4,340,605, U.S. Patent 4,438,141, and U.S. Patent No. 4,444,779 (the "'779 Patent"). The '779 patent is of particular relevance in this appeal and is discussed below. *Takeda*, 417 F.Supp.2d at 378.

The court next considered an article that was published the following year in 1982 by T. Sodha et al. entitled "Studies on Antidiabetic Agents. II. Synthesis of 5–[4–(1–Methylcyclohexylmethoxy)–benzyl–]thiazolidine–2,4–dione (ADD–3878) and Its Derivatives" ("Sodha II"). The Sodha II reference disclosed data relating to hypoglycemic activity and plasma triglyceride lowering activity for 101 TZD compounds. Those compounds did not include pioglitazone, but included compound b. Significantly, Sodha II identified three specific compounds that were deemed most favorable in terms of toxicity and activity. Notably, compound b was not identified as one of the three most favorable compounds. On the contrary, compound b, was singled out as causing "considerable increases in body weight and brown fat weight."

The court also considered Takeda's '779 patent. That patent covers a subset of compounds originally included in the '200 patent application, namely, TZD compounds "where the pyridyl or thiazolyl groups may be substituted." *Id.* at 353. The broadest claim of the '779 patent covers over one million compounds. *Id.* at 378. Compound b was specifically claimed in claim 4 of the patent. The court noted that a preliminary amendment in the prosecution history of the patent contained a statement that "the compounds in which these heterocyclic rings are substituted have become important, especially [compound b]." *Id.*

Based on the prior art as a whole, however, the court found that a person of ordinary skill in the art would not have selected compound b as a lead compound for antidiabetic treatment. Although the prosecution history of the '779 patent included the statement that characterized compound b as "especially important," the court found that any suggestion to select compound b was essentially negated by the disclosure of the Sodha II reference. The court reasoned that one of ordinary skill in the art would not have chosen compound b, notwithstanding the statement in the '779 patent prosecution history, "given the more exhaustive and reliable scientific analysis presented by Sodha II, which taught away from compound b, and the evidence from all of the TZD patents that Takeda filed contemporaneously with the '779 [p]atent showing that there were many promising, broad avenues for further research." *Id.* at 380.

The court found that the three compounds that the Sodha II reference identified as "most favorable" and "valuable for the treatment of maturity-onset diabetes," not compound b, would have served as the best "starting point for further investigation" to a person of ordinary skill in the art. *Id.* at 376. Because diabetes is a chronic disease and thus would require long term treatment, the court reasoned that researchers would have been dissuaded from selecting a lead compound that exhibited negative effects, such as toxicity, or other adverse side effects, especially one that causes "considerable increases in body weight and brown fat weight." *Id.* at 376–77. Thus, the court determined that the prior art did not suggest to one of ordinary skill in the art that compound b would be the best candidate as the lead compound for antidiabetic research.

Admissions from Alphapharm witnesses further buttressed the court's conclusion. Dr. Rosenberg, head of Alphapharm's intellectual property department, testified as a 30(b)(6) witness on behalf of Alphapharm. In discussing Sodha II, Dr. Rosenberg admitted that there was nothing in

the article that would recommend that a person of ordinary skill in the art choose compound b over other compounds in the article that had the same efficacy rating. Dr. Rosenberg, acknowledging that compound b had the negative side effects of increased body weight and brown fat, also admitted that a compound with such side effects would "presumably not" be a suitable candidate compound for treatment of Type II diabetes. Alphapharm's expert, Dr. Mosberg, concurred in that view at his deposition when he admitted that a medicinal chemist would find such side effects "undesirable."

Moreover, another Alphapharm 30(b)(6) witness, Barry Spencer, testified at his deposition that in reviewing the prior art, one of ordinary skill in the art would have chosen three compounds in Sodha II as lead compounds for research, not solely compound b. In addition, Takeda's witness, Dr. Morton, testified that at the time Sodha II was published, it was known that obesity contributed to insulin resistance and Type 2 diabetes. Thus, one of ordinary skill in the art would have concluded that Sodha II taught away from pyridyl compounds because it associated adverse side effects with compound b.

We do not accept Alphapharm's assertion that *KSR*, as well as another case recently decided by this court, *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348 (Fed.Cir. 2007), mandates reversal. Relying on *KSR,* Alphapharm argues that the claimed compounds would have been obvious because the prior art compound fell within "the objective reach of the claim," and the evidence demonstrated that using the techniques of homologation and ring-walking would have been "obvious to try." Additionally, Alphapharm argues that our holding in *Pfizer*, where we found obvious certain claims covering a particular acid-addition salt, directly supports its position.

We disagree. The *KSR* Court recognized that "[w]hen there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp." *KSR,* 127 S.Ct. at 1732. In such circumstances, "the fact that a combination was obvious to try might show that it was obvious under § 103." *Id.* That is not the case here. Rather than identify predictable solutions for antidiabetic treatment, the prior art disclosed a broad selection of compounds any one of which could have been selected as a lead compound for further investigation. Significantly, the closest prior art compound (compound b, the 6–methyl) exhibited negative properties that would have directed one of ordinary skill in the art away from that compound. Thus, this case fails to present the type of situation contemplated by the Court when it stated that an invention may be deemed obvious if it was "obvious to try." The evidence showed that it was not obvious to try.

Similarly, Alphapharm's reliance on *Pfizer* fares no better. In *Pfizer,* we held that certain claims covering the besylate salt of amlodipine would have been obvious. The prior art included a reference, referred to as the Berge reference, that disclosed a genus of pharmaceutically acceptable anions that could be used to form pharmaceutically acceptable acid addition salts, as well as other publications that disclosed the chemical characteristics of the besylate salt. *Pfizer*, 480 F.3d at 1363. Noting that our conclusion was based on the "particularized facts of this case," we found that the prior art provided

"ample motivation to narrow the genus of 53 pharmaceutically-acceptable anions disclosed by Berge to a few, including benzene sulphonate." *Id.* at 1363, 1367. Here, the court found nothing in the prior art to narrow the possibilities of a lead compound to compound b. In contrast, the court found that one of ordinary skill in the art would have chosen one of the many compounds disclosed in Sodha II, of which there were over ninety, that "did not disclose the existence of toxicity or side effects, and to engage in research to increase the efficacy and confirm the absence of toxicity of those compounds, rather than to choose as a starting point a compound with identified adverse effects." Thus, *Pfizer* does not control this case.

Based on the record before us, we conclude that the district court's fact-findings were not clearly erroneous and were supported by evidence in the record. Moreover, we reject the assertion that the court failed to correctly apply the law relating to prima facie obviousness of chemical compounds. Because Alphapharm's obviousness argument rested entirely on the court making a preliminary finding that the prior art would have led to the selection of compound b as the lead compound, and Alphapharm failed to prove that assertion, the court did not commit reversible error by failing to apply a presumption of motivation. We thus conclude that the court did not err in holding that Alphapharm failed to establish a prima facie case of obviousness. *See Eli Lilly & Co. v. Zenith Goldline Pharms.*, 471 F.3d 1369 (Fed.Cir. 2006) (affirming the district court's finding of nonobviousness upon concluding, in part, that the prior art compound would not have been chosen as a lead compound).

b. Choice of the Claimed Compounds

[6] Even if Alphapharm had established that preliminary finding, and we have concluded that it did not, the record demonstrates that Alphapharm's obviousness argument fails on a second ground. The district court found nothing in the prior art to suggest making the specific molecular modifications to compound b that are necessary to achieve the claimed compounds. In reaching that conclusion, the court first found that the process of modifying lead compounds was not routine at the time of the invention. *Takeda*, 417 F.Supp.2d at 380. Dr. Mosberg opined that the steps of homologation and ring-walking were "routine steps in the drug optimization process," but the court found that testimony unavailing in light of the contrary, more credible, testimony offered by Takeda's experts. *Id.* at 381. In addition, the court relied on Dr. Rosenberg's admission that a person of ordinary skill in the art would "look at a host of substituents, such as chlorides, halides and others, not just methyls" in modifying the pyridyl ring. *Id.*

Pioglitazone differs from compound b in two respects, and one would have to both homologate the methyl group of compound b and move the resulting ethyl group to the 5–position on the pyridyl ring in order to obtain pioglitazone. With regard to homologation, the court found nothing in the prior art to provide a reasonable expectation that adding a methyl group to compound b would reduce or eliminate its toxicity. Based on the test results of the numerous compounds disclosed in Sodha II, the court concluded that "homologation had no tendency to decrease unwanted side effects" and thus researchers would have been inclined "to focus research efforts elsewhere." *Id.* at 383. Indeed, several other compounds exhibited similar or better potency than compound b, and one compound in particular, compound 99, that had no identified problems differed signifi-

cantly from compound b in structure. *Id.* at 376 n. 51. Moreover, Dr. Mosberg agreed with Takeda's expert, Dr. Danishefsky, that the biological activities of various substituents were "unpredictable" based on the disclosure of Sodha II. *Id.* at 384–85. The court also found nothing in the '200 and '779 patents to suggest to one of ordinary skill in the art that homologation would bring about a reasonable expectation of success.

As for ring-walking, the court found that there was no reasonable expectation in the art that changing the positions of a substituent on a pyridyl ring would result in beneficial changes. Dr. Mosberg opined that the process of ring-walking was "known" to Takeda, but the court found that testimony inapt as it failed to support a reasonable expectation to one of ordinary skill in the art that performing that chemical change would cause a compound to be more efficacious or less toxic. *Id.* at 382. Moreover, Dr. Mosberg relied on the efficacy data of phenyl compounds in Sodha II, but the court found those data insufficient to show that the same effects would occur in pyridyl compounds.

Alphapharm relies on *In re Wilder,* 563 F.2d 457 (CCPA 1977), for the proposition that differences in a chemical compound's properties, resulting from a small change made to the molecule, are reasonably expected to vary by degree and thus are insufficient to rebut a prima facie case of obviousness. In *Wilder,* our predecessor court affirmed the Board's holding that a claimed compound, which was discovered to be useful as a rubber antidegradant and was also shown to be nontoxic to human skin, would have been obvious in light of its homolog and isomer that were disclosed in the prior art. The evidence showed that the homolog was similarly nontoxic to the human skin, whereas the isomer was toxic. The court held that "one who claims a compound, per se, which is structurally similar to a prior art compound must rebut the presumed expectation that the structurally similar compounds have similar properties." *Id.* at 460. While recognizing that the difference between the isomer's toxicity and the nontoxicity of the homolog and claimed compound "indicate[d] some degree of unpredictability," the court found that the appellant failed to "point out a single actual difference in properties between the claimed compound and the homologue," and thus failed to rebut the presumption. *Wilder,* 563 F.2d at 460.

We would note that since our *Wilder* decision, we have cautioned "that generalization should be avoided insofar as specific chemical structures are alleged to be prima facie obvious one from the other," *Grabiak,* 769 F.2d at 731. In addition to this caution, the facts of the present case differ significantly from the facts of Wilder. Here, the court found that pioglitazone exhibited unexpectedly superior properties over the prior art compound b. *Takeda,* 417 F.Supp.2d at 385. The court considered a report entitled "Preliminary Studies on Toxicological Effects of Ciglitazone–Related Compounds in the Rats" that was presented in February 1984 by Dr. Takeshi Fujita, then-Chief Scientist of Takeda's Biology Research Lab and co-inventor of the '777 patent. That report contained results of preliminary toxicity studies that involved selected compounds, including pioglitazone and compound b. Compound b was shown to be "toxic to the liver, heart and erythrocytes, among other things," whereas pioglitazone was "comparatively potent" and "showed no statistically significant toxicity." *Id.* at 356–57. During the following months, Takeda per-

formed additional toxicity studies on fifty compounds that had been already synthesized and researched by Takeda, including pioglitazone. The compounds were tested for potency and toxicity. The results were presented in another report by Fujita entitled "Pharmacological and Toxicological Studies of Ciglitazone and Its Analogues." Pioglitazone was shown to be the only compound that exhibited no toxicity, although many of the other compounds were found to be more potent. *Id.* at 358.

Thus, the court found that there was no reasonable expectation that pioglitazone would possess the desirable property of nontoxicity, particularly in light of the toxicity of compound b. The court's characterization of pioglitazone's unexpected results is not clearly erroneous. As such, *Wilder* does not aid Alphapharm because, unlike the homolog and claimed compound in *Wilder* that shared similar properties, pioglitazone was shown to differ significantly from compound b, of which it was not a homolog, in terms of toxicity. Consequently, Takeda rebutted any presumed expectation that compound b and pioglitazone would share similar properties.

[7] Alphapharm also points to a statement Takeda made during the prosecution of the '779 patent as evidence that there was a reasonable expectation that making changes to the pyridyl region of compound b would lead to "better toxicity than the prior art." During prosecution of the '779 patent, in response to an enablement rejection, Takeda stated that "there should be no reason in the instant case for the Examiner to doubt that the claimed compounds having the specified substituent would function as a hypolipidemic and hypoglycemic agent as specified in the instant disclosure." That statement, however, indicates only that changes to the left moiety of a lead compound would create compounds with the same properties as the compounds of the prior art; it does not represent that lower toxicity would result. And even if the statement did so represent, it does not refer to any specific substituent at any specific position of TZD's left moiety as particularly promising. As the court correctly noted, the compounds disclosed in the '779 patent included a variety of substituents, including lower alkyls, halogens, and hydroxyl groups, attached to a pyridyl or thiazolyl group. As discussed *supra*, the district court found that the claims encompassed over one million compounds. Thus, we disagree with Alphapharm that that statement provided a reasonable expectation to one of ordinary skill in the art that performing the specific steps of replacing the methyl group of the 6–methyl compound with an ethyl group, and moving that substituent to the 5–position of the ring, would have provided a broad safety margin, particularly in light of the district court's substantiated findings to the contrary.

We thus conclude that Alphapharm's challenges fail to identify grounds for reversible error. The court properly considered the teachings of the prior art and made credibility determinations regarding the witnesses at trial. We do not see any error in the district court's determination that one of ordinary skill in the art would not have been prompted to modify compound b, using the steps of homologation and ring-walking, to synthesize the claimed compounds. Because the court's conclusions are not clearly erroneous and are supported by the record evidence, we find no basis to disturb them.

The court properly concluded that Alphapharm did not make out a prima facie case of obviousness because Alphapharm

failed to adduce evidence that compound b would have been selected as the lead compound and, even if that preliminary showing had been made, it failed to show that there existed a reason, based on what was known at the time of the invention, to perform the chemical modifications necessary to achieve the claimed compounds.

In light of our conclusion that Alphapharm failed to prove that the claimed compounds would have been prima facie obvious, we need not consider any objective indicia of nonobviousness.[5]

2. Scope and Content of the Prior Art

[8] Alphapharm also assigns error to the district court's determination regarding the scope and content of the prior art. Alphapharm asserts that the court excluded the prosecution history of the '779 patent from the scope of the prior art after wrongly concluding that it was not accessible to the public. Takeda responds that the court clearly considered the '779 patent prosecution history, which was admitted into evidence on the first day of testimony. Takeda urges that the court's consideration of the prosecution history is apparent based on its extensive analysis of the '779 patent and the file history that appears in the court's opinion.

We agree with Takeda that the district court did not err in its consideration of the scope of the prior art. As discussed above, the court considered the prosecution history, and even expressly considered one of the key statements in the prosecution history upon which Alphapharm relies in support of its position that compound b would have been chosen as the lead compound. *Takeda*, 417 F.Supp.2d at 378. In considering the prosecution history of the '779 patent, the court noted that Takeda filed a preliminary amendment on March 15, 1983, in which its prosecuting attorney stated that "the compounds in which these heterocyclic rings are substituted have become important, especially [the 6–methyl compound]." *Id.* The court rejected Alphapharm's assertion that that statement supported the conclusion that compound b would have been selected as a lead compound. Rather, the court found that viewing the prior art as a whole, the prior art showed "that Takeda was actively conducting research in many directions, and had not narrowed its focus to compound b." *Id.* at 379. Thus, while the district court may have incorrectly implied that prosecution histories are not accessible to the public, *see id.* at n. 59, *see also Custom Accessories, Inc. v. Jeffrey–Allan Indus.*, 807 F.2d 955 (Fed.Cir.1986) ("[t]he person of ordinary skill is a hypothetical person who is presumed to be aware of all the pertinent prior art"), the court nonetheless considered the prosecution history of the '779 patent in its obviousness analysis and accorded proper weight to the statements contained therein. Thus, any error committed by the court in this regard was harmless error.

We have considered Alphapharm's remaining arguments and find none that warrant reversal of the district court's decision.

5. The concurrence, while agreeing that the question of the "overbreadth" of claims 1 and 5 has been waived, states further that the 6–ethyl compound, which is within the scope of claims 1 and 5, has not been shown to possess unexpected results sufficient to overcome a prima facie case of obviousness, and hence claims 1 and 5 are likely invalid as obvious. Since waiver is sufficient to answer the point being raised, no further comment need be made concerning its substance.

CONCLUSION

We affirm the district court's determination that claims 1, 2, and 5 of the '777 patent have not been shown to have been obvious and hence invalid.

AFFIRMED

Concurring opinion filed by Circuit Judge DYK.

DYK, Circuit Judge, concurring.

I join the opinion of the court insofar as it upholds the district court judgment based on a determination that a claim to pioglitazone (the 5–ethyl compound) would be non-obvious over the prior art. The problem is that only one of the three claims involved here—claim 2—is limited to pioglitazone. In my view, the breadth of the other two claims, claims 1 and 5 of U.S. Patent No. 4,867,777 ("'777 patent")—which are also referenced in the judgment-renders them likely invalid.

All of the compounds claimed in claims 1, 2 and 5 were included in generic claims in the prior art U.S. Patent No. 4,287,200 ("'200 patent"). Unfortunately our law concerning when a species is patentable over a genus claimed in the prior art is less than clear. It is, of course, well established that a claim to a genus does not necessarily render invalid a later claim to a species within that genus. *See Eli Lilly & Co. v. Bd. of Regents of Univ. of Wash.*, 334 F.3d 1264, 1270 (Fed.Cir.2003). In my view a species should be patentable over a genus claimed in the prior art only if unexpected results have been established. Our case law recognizes the vital importance of a finding of unexpected results, both in this context and in the closely related context where a prior art patent discloses a numerical range and the patentee seeks to claim a subset of that range. *See Application of Petering*, 49 C.C.P.A. 993, 301 F.2d 676, 683 (1962) (species found patentable when genus claimed in prior art because unexpected properties of the species were shown); *see also Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1371 (Fed.Cir.2007) (relying on lack of unexpected results in determining that species claim was obvious in view of prior art genus claim); *In re Woodruff*, 919 F.2d 1575, 1578 (Fed.Cir. 1990) (when applicant claims a subset of a range disclosed in a prior art patent, the applicant must generally show that "the claimed range achieves unexpected results relative to the prior art range.").

While the 5–ethyl compound (pioglitazone) is within the scope of the '200 patent, there is clear evidence, as the majority correctly finds, of unexpected results regarding that compound, and therefore its validity is not in question on this ground. However, at oral argument the patentee admitted that the prior art '200 patent also generically covers the 6–ethyl compound, which is within the scope of claims 1 and 5 of the '777 patent, and admitted that there is no evidence of unexpected results for the 6–ethyl compound. Under such circumstances, I believe that the 6–ethyl is likely obvious, and consequently claims 1 and 5 are likely invalid for obviousness. However, the argument as to the overbreadth of claims 1 and 5 has been waived, because it was not raised in the opening brief. In any event, as a practical matter, the judgment finding that the appellants' filing of the ANDA for pioglitazone is an infringement and barring the making of pioglitazone is supported by the finding that claim 2 standing alone is not invalid and is infringed.

REAL PARTY IN INTEREST

The real party in interest in this appeal is Elan Pharma International Limited, which is the assignee of the present application as recorded at Reel/Frame numbers 015165/0833.

RELATED APPEALS AND INTERFERENCES

No related appeals or interferences are pending.

STATUS OF CLAIMS

Claims 1-95 are pending, with claims 32-35, 39, 41-43, and 45-95 withdrawn from consideration. Claims 1-31, 36-38, 40, and 44 are finally rejected, and are the subject of this appeal. The pending claims are presented in Appendix A of this Brief.

STATUS OF AMENDMENTS

No claim amendments were submitted accompanying the response filed on November 5, 2009. No other amendments or submissions are pending in the application.

SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 1 is to be argued in the brief. The relevant citation to the specification is shown in the parentheses below.

Independent claim 1 reads as follows:

1. A nimesulide composition **{p. 1, ll. 7-8}** comprising:

(a) particles of nimesulide **{p. 1, ll. 7-8}** or a salt thereof **{p. 31, ll. 11-13}**, wherein the particles have an effective average particle size of less than 2000 nm **{p. 1, ll. 7-9; p. 6, ll. 16-18}**; and

(b) at least one surface stabilizer adsorbed on the surface of the nimesulide particles **{p. 6, ll. 15-16}**.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The rejections to be reviewed on appeal are the following:

1. Rejection of claims 1-15, and 27-31 under 35 U.S.C. §103(a) for allegedly being obvious over U.S. Patent No. 5,711,961 to Reiner et al. ("Reiner"), in view of U.S. Patent No. 6,375,986 to Ryde et al. ("Ryde");

2. Rejection of claims 1, 10-13, and 15-26 under 35 U.S.C. §103(a) for allegedly being obvious over Reiner and Ryde in view of U.S. Patent No. 5,552,160 to Liversidge et al. ("Liversidge");

3. Rejection of claims 1 and 16-26 under 35 U.S.C. §103(a) for allegedly being obvious over Reiner and Ryde, in view of Singh et al., *Analytical Profiles of Drug Substances and Excipients*, 28: 197-249 (2001) ("Singh") and U.S. Patent No. 5,510,118 to Bosch et al. ("Bosch");

4. Rejection of claims 1, 36-38, and 40 under 35 U.S.C. §103(a) for allegedly being obvious over Reiner and Ryde, in view of Singh and The Merck Index 12th ed., Merck & Co. pp. 416-417 (1996) ("Merck"); and

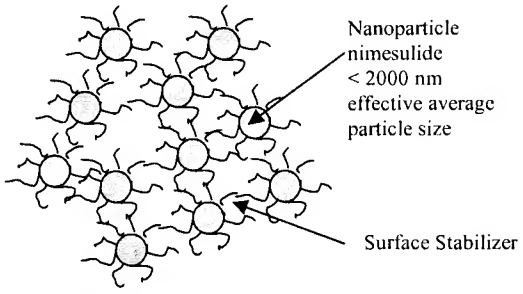
5. Rejection of claims 1 and 44 under 35 U.S.C. §103(a) for allegedly being obvious over Reiner and Ryde, in view of U.S. Patent No. 5,776,563 to Buhl et al. ("Buhl").

ARGUMENT

I. Introduction.

When the particles of an active agent are reduced to a nanoparticulate size range, the small particles are physically attracted to each other and tend to aggregate into larger particles. Therefore, one of the challenges in the nanotechnology field is to maintain the small particle size of the active agent. To obtain a stable nanoparticulate active agent composition, one approach is to adsorb at least one surface stabilizer on the surface of the small particles of the active agent, thereby preventing the small particles from aggregation or agglomeration. It is theorized that the mechanism by which the surface stabilizers prevent aggregation or agglomeration is by steric hindrance.

The claimed invention is directed to steric stabilization of nanoparticles of nimesulide. The presence of a surface stabilizer adsorbed on the surface of the nimesulide nanoparticles acts as a steric barrier to prevent the nimesulide nanoparticles from agglomeration, thereby obtaining a stable nanoparticulate nimesulide composition. The claimed nimesulide composition comprises solid particles of nimesulide having an effective average particle size of less than about 2000 nm, and at least one surface stabilizer adsorbed on the surface of the nimesulide particles. The claimed composition is depicted in the diagram below.



In the prior responses, Appellants consistently argued that one of ordinary skill in the art would not have any reason to modify the chewing gum tablets of Reiner in view of the teachings

of Ryde, and that even if the teachings of the cited references are combined, one of ordinary skill in the art would not have been able to obtain the claimed invention.

As a result, the primary disagreement between Appellants and the Examiner is the ultimate legal determination of obviousness under 35 U.S.C. §103(a). Appellants respectfully request that the Board resolve the disagreement by reversing the rejection in whole for the reasons detailed below.

As noted above, there are five (5) pending rejections of the claims. The first rejection relies on U.S. Patent No. 5,711,961 to Reiner et al. ("Reiner") and U.S. Patent No. 6,375,986 to Ryde et al. ("Ryde"), and the remaining four (4) rejections of the claims rely on these two references and additionally cite the tertiary references identified above. The tertiary references are addressed in Section III below.

II. Rejection over Reiner and Ryde.

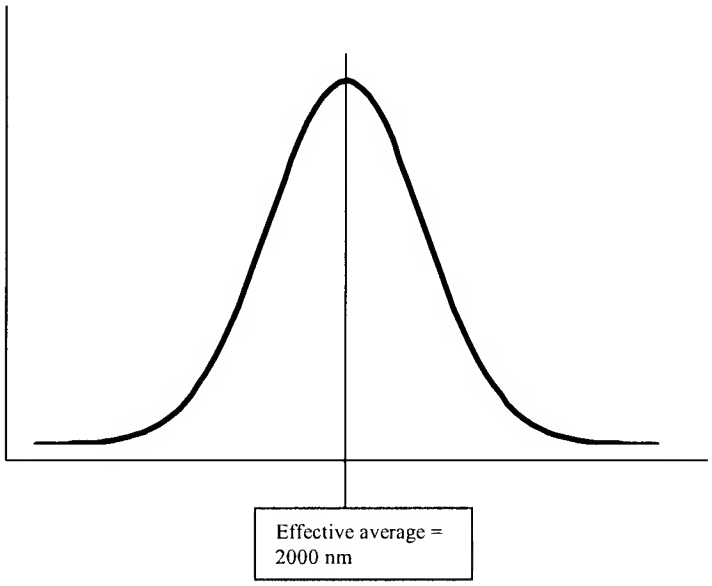
A. Reiner's disclosure of "micron range" particles fails to teach or suggest the claimed particle size.

In the response filed on November 5, 2009, Appellants submitted a detailed analysis as to the difference between the claim recitation of "an effective average particle size of less than 2000 nm" and Reiner's teaching of a particle size "in the micron range." *See* Section II. A. (1) bridging pages 26 and 27. The previously submitted arguments are not repeated but incorporated by reference.

The Examiner asserts that "(a) the drug particles of Reiner are sprayed onto the surface of sugary microgranules having a size of 850 microns, so that by implication, the relative size of the drug particles must be much smaller than the sugary microgranules onto which they are coated, and (b) the plain meaning of 'micronized' connotes 'to pulverize, especially into particles a few micrometers [microns] in diameter.'" Final Office Action dated February 18, 2010.

Concerning point (a), the size of 850 microns of the sugary microgranules does not have any implication on the drug particle size, as the Examiner contends. It is unclear from the disclosure of Reiner, as a whole, that the drug particle size of Reiner's chewing gum falls within the claimed particle size. Assuming, *arguendo*, that some of Reiner's drug particles are "much smaller than the sugary microgranules," there is no indication that Reiner's drug particles have a ***particle size distribution***, where at least 50% of the particles have a size of less than 2000 nm.

In contrast, the claimed invention requires that the nimesulide particles have an effective average particle size of less than 2000 nm. As explicitly defined in the specification, "an effective average particle size of less than 2000 nm" means that at least 50% of the nimesulide particles have a particle size of less than 2000 nm. The nimesulide particle size distribution of Appellants' claims is represented by the bell-shaped curve below:



Accordingly, Reiner's explicit or implicit teaching of particle size fails to render the effective average particle size of the claimed invention obvious.

Regarding point (b), the plain meaning of "micronized," even taken at the face value of "a few microns," as suggested by the Examiner, fails to teach or suggest the effective average

particle size of the claimed invention. As discussed above, there is no teaching or suggestion of a particle size distribution which can be gleaned from Reiner's disclosure.

Moreover, nanoparticles, as defined by online Wikipedia (http://en.wikipedia.org/wiki/Nanoparticle), encompass fine particles, which cover a size range between 100 and 2500 nm, and ultrafine particles, which cover a size range between 1 and 100 nm. Reiner's teaching of "micronized" particles or "micron range," even when interpreted to mean "a few microns," falls largely outside of the population of nanoparticles.

For these reasons alone, the Examiner has failed to establish a *prima facie* case of obviousness.

B. A reason to combine the teachings of Reiner and Ryde is lacking.

The Examiner further relied on the secondary reference, Ryde, for the alleged teaching of an effective average particle size according to Appellants' claims. However, one of ordinary skill in the art would not have any reason to modify Reiner's teaching by reducing the drug particle size in view of Ryde's teaching. The arguments submitted on November 5, 2009 are incorporated herewith by reference.

The Examiner contends that "both Reiner and Ryde disclose solid-dose nanoparticle pharmaceutical compositions formulated for oral administration of poorly soluble active agents," and that "[i]mproving a drug's pharmacokinetic profile, such as its dispersibility and bioavailability, is a common objective in the pharmaceutical arts, which can be achieved by making one or more of several well-known modifications." Final Office Action, page 5, lines 13-17. The Examiner's analyses and conclusions are incorrect.

First, the Examiner's interpretation of Reiner and Ryde is technically incorrect. Reiner's chewing gums comprising "micronized" drug particles are not nanoparticle compositions, as discussed *supra*. Second, Reiner's chewing gums are administered orally; while Ryde's

compositions are not limited to oral administration but can be formulated for rectal, pulmonary, intravaginal, or local administration, or be formulated as a buccal or nasal spray. *See* Ryde, column 10, lines 48-50.

Accordingly, Reiner and Ryde have different goals of solving different problems. More specifically, Reiner aims at preparing a chewing gum as a delivery vehicle for active agents. Column 1, lines 7-8. As such, Reiner focuses on achieving a palatable taste when the drug is released during the chewing process by using a particular lacquering system. *Id.*, lines 16-22. Not surprisingly, Reiner is silent about the redispersibility of the drug because once the drug is made into a chewing gum, there is no need to redisperse the drug.

In contrast, Ryde unexpectedly discovered that nanoparticulate active agent compositions achieved superior redispersibility when at least one polymeric surface stabilizer and dioctyl sodium sulfosuccinate (DOSS) are used. *See* the abstract. Ryde further elaborates that DOSS or polymeric stabilizers achieved synergistic effect in redispersibility because neither one was able to achieve the redisperse profile alone. *See* column 5, lines 60-67.

Based on the prior art teachings, one of ordinary skill in the art would not have had any reason to combine the references because there is no correlation established between palatability of a chewing gum and redispersibility of a nanoparticulate active agent composition.

The Examiner's comment that "[i]mproving a drug's pharmacokinetic profile, such as its dispersibility and bioavailability, is a common objective in the pharmaceutical art" is an overly generalized statement and lacks support from the cited reference. Although it is desirable that all pharmaceutical compositions have superior redispersibility and bioavailability, there is no evidence that these properties rather than palatability are the major concern of Reiner's chewing gum. Moreover, Reiner is silent regarding any further particle size reduction to nanoparticulate particles. By the same token, one skilled in the art informed by the teachings of Ryde would not have modified Reiner's chewing gum by reducing the drug particle size because Reiner has no

suggestion that improved redispersibility would have benefited the chewing gum dosage form. As such, the Examiner could only have made the rejection with the aid of impermissible hindsight.

The Examiner denies that impermissible hindsight was applied because Ryde recognizes the problem of poor redispersibility of nanoparticulate active agent compositions and aims to improve redispersibility of such compositions. *See* final Office Action, page 6, 1st and 2nd paragraphs. This acknowledgement does not provide any reason to combine the teachings of Ryde and Reiner, however. This is because Reiner is irrelevant to a nanoparticulate active agent composition, and Reiner's chewing gum has a palatability problem but not a redispersibility problem. Therefore, in the absence of any correlation between palatability of a chewing gum comprising micronized drug particles and redispersibility of a nanoparticulate active agent composition, the Examiner has failed to provide a reasonable rationale to substantiate the rejection.

C. The Examiner fails to articulate a reasonable expectation of success to support the rejection rationale.

The Examiner asserts that "optimizing the particle size and choice of excipient(s) involves routine experimentation rather than an inventive step, particularly when the adjustments have been attempted and proven useful in similar drug formulations, as in Reiner and Ryde" (final Office Action, page 5, last 3 lines). As discussed in the foregoing paragraphs, Reiner and Ryde do ***not*** disclose similar drug formulations. Moreover, at the time of filing the present application, it was unknown whether a stable nanoparticulate nimesulide composition could be obtained. Therefore, the issue is not whether optimizing the particle size is a routine experimentation. Rather, the issue is to determine which active agent can produce a stable nanoparticulate active agent composition. At most, the Examiner attempts an obvious-to-try rationale to support the rejection.

The Examination Guidelines for Determining Obviousness under 35 U.S.C. § 103 in view of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.* ("the Guidelines") requires the Examiner to articulate the following when making a rejection based on an obvious-to-try rationale:

(1) a finding that at the time of the invention, there had been a recognized problem or need in the art, which may include a design need or market pressure to solve a problem;

(2) a finding that there had been a finite number of identified, predictable potential solutions to the recognized need or problem;

(3) a finding that one of ordinary skill in the art could have pursued the known potential solutions with a reasonable expectation of success; and

(4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

In the present case, Ryde does not disclose a ***finite number*** of ***identified, predictable*** potential active agents to be used in nanoparticulate active agent compositions. Rather, Ryde discloses over 40 ***categories*** of active agents, and each category encompasses an enormous number of compounds. *See* Ryde, column 7, lines 1-23. Reiner discloses a chewing gum, in which the drug is in a micron size range but fails to disclose any nanoparticulate active agent composition.

Even if nimesulide falls within a subgenus of the drugs disclosed by Ryde, one of ordinary skill in the art would have to try each species of the subgenus until a nanoparticulate active agent composition could be successfully obtained, in the absence of any knowledge that a stable nanoparticulate nimesulide composition could be made.

A suggestion to try each species of the subgenus is explicitly rejected as a proper application of "obvious to try" rationale in the recent Federal Circuit ruling, excerpted below:

> *To differentiate between proper and improper applications of "obvious to try," this court outlined two classes of situations where* ***"obvious to try" is erroneously equated with obviousness under §103****. In the first class of cases, what would have been "obvious to try" would have been to vary all parameters or* ***try each of numerous possible choices*** *until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.*

In re Kubin, 561 F.3d 1351, 1359 (Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988); (emphasis added). *Kubin* further analogizes this rejection rationale with "throw[ing] metaphorical darts at a board filled with combinatorial prior art possibilities" with the aid of hindsight, and therefore, is improper. *Id.*

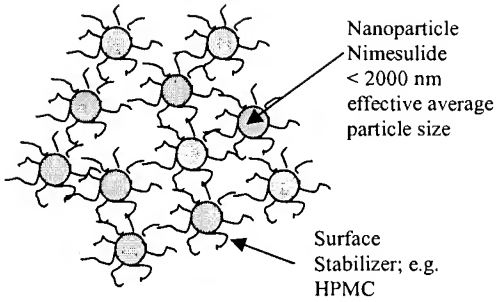
This point has been affirmed by *Takeda Chemical Industries v. Alphapharm Pty.* 492 F.3d 1350 (Fed. Cir. 2007). In *Takeda*, the prior art discloses "hundreds of millions of TZD compounds" and specifically identifies 54 compounds, including compound b. The court found it non-obvious to select compound b, however, because there was no indication in the prior art to show that compound b fell in the group of "the best performing compounds." *Id.* at 1357.

Accordingly, the Examiner's rejection rationale is inconsistent with the Examination Guidelines or the court rulings.

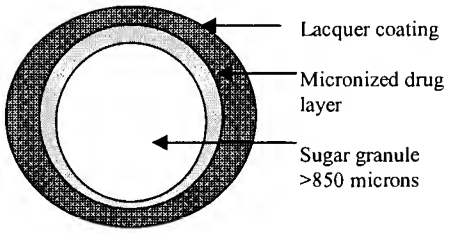
D. Even if the teachings of Reiner and Ryde are combined, one of ordinary skill in the art would not have been able to obtain the claimed invention.

In the response filed on November 5, 2009, Appellants argued that modification of Reiner's chewing gum in view of Ryde's teaching would have destroyed the intended purpose of the lacquer layer of Reiner's gum. *See* Section II A (3) spanning pages 30 and 31, which is incorporated by reference.

As submitted in a prior response filed on March 9, 2009, the claimed invention and Reiner's chewing gum are depicted in the diagrams below:



(A) claimed composition



(B) Reiner's chewing gum

The claimed composition comprises nimesulide nanoparticles and at least one surface stabilizer adsorbed on the surface of nimesulide particles. The nimesulide particles are not coated, encapsulated, or isolated from the surrounding environment. In contrast, Reiner's chewing gum has a lacquer coating to encapsulate the micronized drug layer. One of ordinary skill in the art would not have any reason to modify Reiner's chewing gum in view of Ryde's teaching in an attempt to obtain the claimed composition because the proposed modification would require removal of the lacquer coating, which would destroy the intended purpose of Reiner's chewing gum.

Alternatively, if the Examiner suggests that one skilled in the art could add a surface stabilizer into the micronized drug layer, then the modification would not achieve the claimed composition, which is free of any lacquer coating. Moreover, the prior art does not provide any reason to add a surface stabilizer to the micronized drug. Reiner does not teach or suggest that the micronized drug having a particle size in the micron range is unstable and requires any steric stabilization.

III. Rejection over Reiner, Ryde and Tertiary References.

The foregoing discussions concerning Reiner and Ryde are incorporated by reference. The Examiner relies on a number of tertiary references for allegedly meeting the limitations of certain dependent claims. For instance, Liversidge Singh and Bosh are cited for the alleged

teaching of pharmacokinetic profiles, T_{max}, C_{max} and AUC, of the claimed nanoparticulate nimesulide composition. Merck is cited for the alleged teaching that a secondary active agent, codeine, has analgesic properties. Finally, Buhl is cited for the alleged teaching of sterile filtration.

Because none of the tertiary references compensate for the deficiencies of Reiner and Ryde, as discussed above, the claims at issue are non-obvious for depending from a non-obvious base claim.

Accordingly, Appellants respectfully request that the Board reverse the rejection under 35 U.S.C. §103(a) in whole.

CONCLUSION

For the reasons discussed above, Appellants respectfully submit that all pending claims are in condition for allowance, and respectfully request that the rejections be reversed in whole, and that the claims be allowed to issue.

Respectfully submitted,

Date: July 29, 2010

By /Michele M. Simkin/

FOLEY & LARDNER LLP
Customer Number: 31049
Telephone: (202) 672-5538
Facsimile: (202) 672-5399

Michele M. Simkin
Attorney for Applicant
Registration No. 34,717

APPENDIX A: CLAIMS INVOLVED IN APPEAL

1. (Previously Presented) A nimesulide composition comprising:

(a) particles of nimesulide or a salt thereof, wherein the particles have an effective average particle size of less than 2000 nm; and

(b) at least one surface stabilizer adsorbed on the surface of the nimesulide particles.

2. (Previously Presented) The composition of claim 1, wherein the nimesulide is selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

3. (Previously Presented) The composition of claim 1, wherein the effective average particle size of the nimesulide particles is selected from the group consisting of less than 1900 nm, less than 1800 nm, less than 1700 nm, less than 1600 nm, less than 1500 nm, less than 1400 nm, less than 1300 nm, less than 1200 nm, less than 1100 nm, less than 1000 nm, less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 100 nm, less than 75 nm, and less than 50 nm.

4. (Original) The composition of claim 1, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

5. (Original) The composition of claim 1 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

6. (Original) The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

7. (Original) The composition of claim 1, wherein the nimesulide or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

8. (Original) The composition of claim 1, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

9. (Original) The composition of claim 1, comprising two or more surface stabilizers.

10. (Previously Presented) The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, a non-ionic surface stabilizer, and an ionic surface stabilizer.

11. (Original) The composition of claim 10, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate,

carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone.

12. (Original) The composition of claim 10, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

13. (Previously Presented) The composition of claim 10, wherein the surface stabilizer is selected from the group consisting of cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide,

C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride, lauryl dimethyl (ethenoxy)$_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

14. (Original) The composition of claim 1, comprising as a surface stabilizer a random copolymer of vinyl acetate and vinyl pyrrolidone, hydroxypropylmethyl cellulose, or tyloxapol.

15. (Original) The composition of any of claims 10, 12, or 13, wherein the composition is bioadhesive.

16. (Original) The composition of claim 1, wherein the T_{max} of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

17. (Previously Presented) The composition of claim 16, wherein the T_{max} is selected from the group consisting of not greater than 90%, not greater than 80%, not greater than 70%, not greater than 60%, not greater than 50%, not greater than 30%, not greater than 25%, not greater than 20%, not greater than 15%, and not greater than 10% of the T_{max}, exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

18. (Original) The composition of claim 1, wherein the C_{max} of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

19. (Previously Presented) The composition of claim 18, wherein the C_{max} is selected from the group consisting of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, and at least 100% greater than the C_{max} exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

20. (Original) The composition of claim 1, wherein the AUC of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

21. (Previously Presented) The composition of claim 20, wherein the AUC is selected from the group consisting of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, and at least 100% greater than the AUC exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

22. (Previously Presented) The composition of claim 1 which does not produce a difference in the absorption levels of the nimesulide composition when administered to a patient under fed as compared to fasting conditions.

23. (Previously Presented) The composition of claim 22, wherein the difference in absorption of the nimesulide composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, and less than 3%.

24. (Original) The composition of claim 1, wherein administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state, when administered to a human.

25. (Original) The composition of claim 24, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC, when administered to a human.

26. (Original) The composition of claim 24, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}, when administered to a human.

27. (Previously Presented) The composition of claim 1, further comprising at least one additional nimesulide composition having an effective average particle size which is different than the effective average particle size of the nimesulide composition of claim 1.

28. (Previously Presented) The composition of claim 1, wherein upon administration the composition redisperses such that the nimesulide particles have an effective average particle size of less than 2000 nm.

29. (Previously Presented) The composition of claim 28, wherein upon administration the composition redisperses such that the nimesulide particles have an effective average particle size selected from the group consisting of less than 1900 nm, less than 1800 nm, less than 1700 nm, less than 1600 nm, less than 1500 nm, less than 1400 nm, less than 1300 nm, less than 1200 nm, less than 1100 nm, less than 1000 nm, less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 150 nm, less than 100 nm, less than 75 nm, and less than 50 nm.

30. (Previously Presented) The composition of claim 1, wherein the composition redisperses in a biorelevant media such that the nimesulide particles have an effective average particle size of less than 2 microns.

31. (Previously Presented) The composition of claim 30, wherein the composition redisperses in a biorelevant media such that the nimesulide particles have an effective average particle size selected from the group consisting of less than 1900 nm, less than 1800 nm, less than 1700 nm, less than 1600 nm, less than 1500 nm, less than 1400 nm, less than 1300 nm, less than 1200 nm, less than 1100 nm, less than 1000 nm, less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 150 nm, less than 100 nm, less than 75 nm, and less than 50 nm.

32. (Withdrawn) The composition of claim 1 formulated into a liquid dosage form, wherein the dosage form has a viscosity of less than 2000 mPa·s, measured at 20°C, at a shear rate of 0.1 (1/s).

33. (Withdrawn) The composition of claim 32, having a viscosity at a shear rate of 0.1 (1/s) selected from the group consisting of from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

34. (Withdrawn) The composition of claim 32, wherein the viscosity of the dosage form is selected from the group consisting of less than 1/200, less than 1/100, less than 1/50, less than 1/25, and less than 1/10 of the viscosity of a liquid dosage form of conventional non-nanoparticulate nimesulide at about the same concentration per ml of nimesulide.

35. (Withdrawn) The composition of claims 32, wherein the viscosity of the dosage form is selected from the group consisting of less than 5%, less than 10%, less than 15%, less than 20%, less than 25%, less than 30%, less than 35%, less than 40%, less than 45%, less than 50%, less than 55%, less than 60%, less than 65%, less than 70%, less than 75%, less than 80%,

less than 85%, and less than 90% of the viscosity of a liquid dosage form of conventional, non-nanoparticulate nimesulide at about the same concentration per ml of nimesulide.

36. (Original) The composition of claim 1, additionally comprising one or more non-nimesulide active agents.

37. (Previously Presented) The composition of claim 36, wherein said non-nimesulide active agent is selected from the group consisting of an analgesic, an anti-inflammatory, an antipyretic, and a vasomodulator.

38. (Original) The composition of claim 36, wherein said non-nimesulide active agent is selected from the group consisting of nutraceuticals, proteins, peptides, nucleotides, amino acids, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, NSAIDs, non-nimesulide COX-2 inhibitors, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, vasomodulators, and xanthines.

39. (Withdrawn) The composition of claim 38, wherein said nutraceutical is selected from the group consisting of lutein, folic acid, fatty acids, fruit extracts, vegetable extracts, vitamin supplements, mineral supplements, phosphatidylserine, lipoic acid, melatonin,

glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish oils, marine animal oils, and probiotics.

40. (Original) The composition of claim 36, wherein said non-nimesulide active agent is selected from the group consisting of aceclofenac, acemetacin, e-acetamidocaproic acid, acetaminophen, acetaminosalol, acetanilide, acetylsalicylic acid, S-adenosylmethionine, alclofenac, alfentanil, allylprodine, alminoprofen, aloxiprin, alphaprodine, aluminum bis(acetylsalicylate), amfenac, aminochlorthenoxazin, 3-amino-4-hydroxybutyric acid, 2-amino-4-picoline, aminopropylon, aminopyrine, amixetrine, ammonium salicylate, ampiroxicam, amtolmetin guacil, anileridine, antipyrine, antipyrine salicylate, antrafenine, apazone, bendazac, benorylate, benoxaprofen, benzpiperylon, benzydamine, benzylmorphine, bermoprofen, bezitramide, α-bisabolol, bromfenac, p-bromoacetanilide, 5-bromosalicylic acid acetate, bromosaligenin, bucetin, bucloxic acid, bucolome, bufexamac, bumadizon, buprenorphine, butacetin, butibufen, butophanol, calcium acetylsalicylate, carbamazepine, carbiphene, carprofen, carsalam, chlorobutanol, chlorthenoxazin, choline salicylate, cinchophen, cinmetacin, ciramadol, clidanac, clometacin, clonitazene, clonixin, clopirac, clove, codeine, codeine methyl bromide, codeine phosphate, codeine sulfate, cropropamide, crotethamide, desomorphine, dexoxadrol, dextromoramide, dezocine, diampromide, diclofenac sodium, difenamizole, difenpiramide, diflunisal, dihydrocodeine, dihydrocodeinone enol acetate, dihydromorphine, dihydroxyaluminum acetylsalicylate, dimenoxadol, dimepheptanol, dimethylthiambutene, dioxaphetyl butyrate, dipipanone, diprocetyl, dipyrone, ditazol, droxicam, emorfazone, enfenamic acid, epirizole, eptazocine, etersalate, ethenzamide, ethoheptazine, ethoxazene, ethylmethylthiambutene, ethylmorphine, etodolac, etofenamate, etonitazene, eugenol, felbinac, fenbufen, fenclozic acid, fendosal, fenoprofen, fentanyl, fentiazac, fepradinol, feprazone, floctafenine, flufenamic acid, flunoxaprofen, fluoresone, flupirtine, fluproquazone, flurbiprofen, fosfosal, gentisic acid, glafenine, glucametacin, glycol salicylate, guaiazulene, hydrocodone, hydromorphone, hydroxypethidine, ibufenac, ibuprofen, ibuproxam, imidazole salicylate,

indomethacin, indoprofen, isofezolac, isoladol, isomethadone, isonixin, isoxepac, isoxicam, ketobemidone, ketoprofen, ketorolac, p-lactophenetide, lefetamine, levorphanol, lofentanil, lonazolac, lomoxicam, loxoprofen, lysine acetylsalicylate, magnesium acetylsalicylate, meclofenamic acid, mefenamic acid, meperidine, meptazinol, mesalamine, metazocine, methadone hydrochloride, methotrimeprazine, metiazinic acid, metofoline, metopon, mofebutazone, mofezolac, morazone, morphine, morphine hydrochloride, morphine sulfate, morpholine salicylate, myrophine, nabumetone, nalbuphine, 1-naphthyl salicylate, naproxen, narceine, nefopam, nicomorphine, nifenazone, niflumic acid, nimesulide, 5'-nitro-2'-propoxyacetanilide, norlevorphanol, normethadone, normorphine, norpipanone, olsalazine, opium, oxaceprol, oxametacine, oxaprozin, oxycodone, oxymorphone, oxyphenbutazone, papaveretum, paranyline, parsalmide, pentazocine, perisoxal, phenacetin, phenadoxone, phenazocine, phenazopyridine hydrochloride, phenocoll, phenoperidine, phenopyrazone, phenyl acetylsalicylate, phenylbutazone, phenyl salicylate, phenyramidol, piketoprofen, piminodine, pipebuzone, piperylone, piprofen, pirazolac, piritramide, piroxicam, pranoprofen, proglumetacin, proheptazine, promedol, propacetamol, propiram, propoxyphene, propyphenazone, proquazone, protizinic acid, ramifenazone, remifentanil, rimazolium metilsulfate, salacetamide, salicin, salicylamide, salicylamide o-acetic acid, salicylsulfuric acid, salsalte, salverine, simetride, sodium salicylate, sufentanil, sulfasalazine, sulindac, superoxide dismutase, suprofen, suxibuzone, talniflumate, tenidap, tenoxicam, terofenamate, tetrandrine, thiazolinobutazone, tiaprofenic acid, tiaramide, tilidine, tinoridine, tolfenamic acid, tolmetin, tramadol, tropesin, viminol, xenbucin, ximoprofen, zaltoprofen, and zomepirac.

41. (Withdrawn) The composition of claim 38, in which the vasomodulator is selected from the group consisting of caffeine, theobromine, and theophylline.

42. (Withdrawn) The composition of claim 38, in which the NSAID is selected from the group consisting of nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, dapsone, aspirin, diflunisal, benorylate, fosfosal, diclofenac, alclofenac,

fenclofenac, etodolac, indomethacin, sulindac, tolmetin, fentiazac, tilomisole, carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen, flufenamic, mefenamic, meclofenamic, niflumic, oxyphenbutazone, phenylbutazone, apazone, feprazone, piroxicam, sudoxicam, isoxicam, and tenoxicam.

43. (Withdrawn) The composition of claim 38, in which the COX-2 inhibitor is selected from the group consisting of celecoxib, rofecoxib, meloxicam, valdecoxib, parecoxib, etoricoxib, SC-236, NS-398, SC-58125, SC-57666, SC-558, SC-560, etodolac, DFU, monteleukast, L-745337, L-761066, L-761000, L-748780, DUP-697, PGV 20229, iguratimod, BF 389, CL 1004, PD 136005, PD 142893, PD 138387, PD 145065, flurbiprofen, nabumetone, flosulide, piroxicam, diclofenac, lumiracoxib, D 1367, R 807, JTE-522, FK-3311, FK 867, FR 140423, FR 115068, GR 253035, RWJ 63556, RWJ 20485, ZK 38997, S 2474, zomepirac analogs, RS 104894, SC 41930, pranlukast, SB 209670, and APHS

44. (Original) The composition of claim 1, which has been sterile filtered.

45. (Withdrawn) A method of making a nimesulide composition comprising contacting particles of nimesulide or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a nimesulide composition having an effective average particle size of less than 2000 nm, wherein the at least one surface stabilizer is adsorbed on the surface of the nimesulide particles.

46. (Withdrawn) The method of claim 45, wherein said contacting comprises grinding.

47. (Withdrawn) The method of claim 46, wherein said grinding comprises wet grinding.

48. (Withdrawn) The method of claim 45, wherein said contacting comprises homogenizing.

49. (Withdrawn) The method of claim 45, wherein said contacting comprises:

(a) dissolving the particles of nimesulide or a salt thereof in a solvent;

(b) adding the resulting nimesulide solution to a solution comprising at least one surface stabilizer; and

(c) precipitating the solubilized nimesulide having at least one surface stabilizer adsorbed on the surface thereof by the addition thereto of a non-solvent.

50. (Withdrawn) The method of claim 45, wherein the nimesulide or a salt thereof is selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

51. (Withdrawn) The method of claim 45, wherein the effective average particle size of the nimesulide particles is selected from the group consisting of less than 1900 nm, less than 1800 nm, less than 1700 nm, less than 1600 nm, less than 1500 nm, less than 1000 nm, less than 1400 nm, less than 1300 nm, less than 1200 nm, less than 1100 nm, less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 100 nm, less than 75 nm, and less than 50 nm.

52. (Withdrawn) The method of claim 45, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

53. (Withdrawn) The method of claim 45, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

54. (Withdrawn) The method of claim 45, wherein the nimesulide or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

55. (Withdrawn) The method of claim 45, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

56. (Withdrawn) The method of claim 45, comprising at two surface stabilizers.

57. (Withdrawn) The method of claim 45, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, a non-ionic surface stabilizer, and an ionic surface stabilizer.

58. (Withdrawn) The method of claim 57, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and

formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

59. (Withdrawn) The method of claim 57, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

60. (Withdrawn) The method of claim 57, wherein the surface stabilizer is selected from the group consisting of cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride, lauryl dimethyl

(ethenoxy)$_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

61. (Withdrawn) The method of claim 45, utilizing as a surface stabilizer a random copolymer of vinyl acetate and vinyl pyrrolidone, hydroxypropylmethyl cellulose, or tyloxapol.

62. (Withdrawn) The method of any of claims 57, 59, or 60, wherein the composition is bioadhesive.

63. (Withdrawn) A method of treating a subject in need comprising administering to the subject an effective amount of a composition comprising:

(a) particles of nimesulide or a salt thereof, wherein the nimesulide particles have an effective average particle size of less than 2000 nm; and

(b) at least one surface stabilizer adsorbed on the surface of the nimesulide particles.

64. (Withdrawn) The method of claim 63, wherein the nimesulide or a salt thereof is selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

65. (Withdrawn) The method of claim 63, wherein the effective average particle size of the nimesulide particles is selected from the group consisting of less than 1900 nm, less than 1800 nm, less than 1700 nm, less than 1600 nm, less than 1500 nm, less than 1400 nm, less than 1300 nm, less than 1200 nm, less than 1100 nm, less than 1000 nm, less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 100 nm, less than 75 nm, and less than 50 nm.

66. (Withdrawn) The method of claim 63, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

67. (Withdrawn) The method of claim 63, wherein the composition is a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

68. (Withdrawn) The method of claim 63, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

69. (Withdrawn) The method of claim 63, wherein the nimesulide or a salt thereof is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

70. (Withdrawn) The method of claim 63, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the nimesulide or a salt thereof and at least one surface stabilizer, not including other excipients.

71. (Withdrawn) The method of claim 63, comprising at two surface stabilizers.

72. (Withdrawn) The method of claim 63, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, a non-ionic surface stabilizer, and an ionic surface stabilizer.

73. (Withdrawn) The method of claim 72, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and

formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

74. (Withdrawn) The method of claim 72, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

75. (Withdrawn) The method of claim 72, wherein the surface stabilizer is selected from the group consisting of benzalkonium chloride, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, cationic lipids, sulfonium compounds, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl

dimethyl (ethenoxy)$_4$ ammonium chloride, lauryl dimethyl (ethenoxy)$_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

76. (Withdrawn) The method of claim 63, utilizing as a surface stabilizer a random copolymer of vinyl acetate and vinyl pyrrolidone, hydroxypropylmethyl cellulose, or tyloxapol.

77. (Withdrawn) The method of any of claims 72, 74, or 75, wherein the composition is bioadhesive.

78. (Withdrawn) The method of claim 63, wherein administration of the nimesulide composition does not produce a difference in the absorption levels of the nimesulide composition when administered to a patient under fed as compared to fasting conditions, when administered to a human.

79. (Withdrawn) The method of claim 78, wherein the difference in absorption of the nimesulide composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, and less than 3%.

80. (Withdrawn) The method of claim 63, wherein administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state, when administered to a human.

81. (Withdrawn) The method of claim 80, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC, when administered to a human.

82. (Withdrawn) The method of claim 80, wherein "bioequivalency" is established by a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}, when administered to a human.

83. (Withdrawn) The method of claim 63, wherein the T_{max} of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

84. (Withdrawn) The method of claim 83, wherein the T_{max} is selected from the group consisting of not greater than 90%, not greater than 80%, not greater than 70%, not greater than 60%, not greater than 50%, not greater than 30%, not greater than 25%, not greater than 20%, not greater than 15%, and not greater than 10% of the T_{max}, exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

85. (Withdrawn) The method of claim 63, wherein the C_{max} of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

86. (Withdrawn) The method of claim 85, wherein the C_{max} is selected from the group consisting of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, and at least 100% greater than the C_{max} exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

87. (Withdrawn) The method of claim 63, wherein the AUC of the nimesulide, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a conventional, non-nanoparticulate form of nimesulide, administered at the same dosage.

88. (Withdrawn) The method of claim 87, wherein the AUC is selected from the group consisting of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, and at least 100% greater than the AUC exhibited by a non-nanoparticulate formulation of nimesulide, administered at the same dosage.

89. (Withdrawn) The method of claim 63, additionally comprising administering one or more non-nimesulide active agents.

90. (Withdrawn) The method of claim 63, additionally comprising administering one or more non-nimesulide active agents effective for treating fever, inflammation or pain.

91. (Withdrawn) The method of claim 89, wherein said non-nimesulide active agent is selected from the group consisting of an analgesic, an anti-inflammatory, an antipyretic, and a vasomodulator.

92. (Withdrawn) The method of claim 89, wherein said non-nimesulide active agent is selected from the group consisting of nutraceuticals, proteins, peptides, nucleotides, amino acids, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, NSAIDs, non-nimesulide COX-2 inhibitors, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, vasomodulators, and xanthines.

93. (Withdrawn) The method of claim 63, wherein the subject is a human.

94. (Withdrawn) The method of claim 63, wherein the method is used to treat a condition selected from the group consisting of rheumatic and joint diseases, arthritis, rheumatoid arthritis, osteoarthritis, periarthritis, tendonitis, bursitis, ankylosing spondylitis, joint stiffness, lower back pain, gynecological conditions, menstrual migraine attack, dysmenorrhoea, pelvic inflammatory disease, urological conditions, urethritis, prostatitis, and vesiculitis pyrexia, cardiovascular diseases, atherosclerosis, hypotension, thrombophlebitis, arthrosis; inflammatory conditions, otitis, rhinitis, sinusitis, pharyngitis, bronchitis nephrotoxicity, mastitis, asthma,

cancer, trauma, surgery, migraine headaches, kidney disease, Alzheimer's disease, familial adenomatous polyposis, diarrhea, colonic adenomas bone resorption, and related conditions.

95. (Withdrawn) The method of claim 63, wherein the method is used to treat a condition where anti-inflammatory agents, anti-angiogenesis agents, antitumorigenic agents, immunosuppressive agents, NSAIDs, COX-2 inhibitors, analgesic agents, anti-thrombotic agents, narcotics, or antifebrile agents are typically used.

APPENDIX B: EVIDENCE

1. U.S. Patent No. 5,711,961 to Reiner et al.;

2. U.S. Patent No. 6,375,986 to Ryde et al.;

3. U.S. Patent No. 5,552,160 to Liversidge et al.;

4. Singh et al., *Analytical Profiles of Drug Substances and Excipients*, 28: 197-249 (2001);

5. U.S. Patent No. 5,510,118 to Bosch et al.;

6. The Merck Index 12th ed., Merck & Co. pp. 416-417 (1996); and

7. U.S. Patent No. 5,776,563 to Buhl et al.

1. U.S. Patent No. 5,711,961 to Reiner et al.

US005711961A

United States Patent [19]

Reiner et al.

[11] Patent Number: 5,711,961

[45] Date of Patent: Jan. 27, 1998

[54] **PHARMACEUTICAL COMPOSITIONS BASED ON CHEWING GUM AND A METHOD FOR THE PREPARATION THEREOF**

[75] Inventors: **Alberto Reiner**, Como; **Alessandro Seneci**, Milan, both of Italy

[73] Assignee: **APR Applied Pharma Research S.A.**, Stabio, Switzerland

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[52] **U.S. Cl.** **424/441**; 424/440; 426/5; 426/3

[58] **Field of Search** 424/440, 441; 426/5, 3

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Primary Examiner—Thurman K. Page
Assistant Examiner—D. Faulkner
Attorney, Agent, or Firm—Nixon & Vanderhye

[57] **ABSTRACT**

Chewing gum tablets and their methods of preparation are disclosed. The gum tablets contain a mixture of chewing gum base and sugary microgranules with an additive agent and an active ingredient adsorbed onto their surface. A lacquer coating on the tablet contains cellulose and polyethlene glycols. The sugary microgranules are delayed release coated particles. The chewing gums act as vehicles for active ingredients. These active ingredients may be used alone or in combination in normal physical form in the form of coated microspheres.

43 Claims, No Drawings

PHARMACEUTICAL COMPOSITIONS BASED ON CHEWING GUM AND A METHOD FOR THE PREPARATION THEREOF

This application is a 371 of PCT/EP95/02816 Jul. 15, 1995.

The subject of the present invention is the preparation of chewing gums which act as vehicles for active ingredients. These active ingredients may be used alone or in combination, in their normal physical form or in the form of coated microspheres.

In a gum there may therefore be various combinations of an active ingredient in its physical form and of the same active ingredient converted into coated microspheres, in various ratios.

More specifically, the subject of the invention is the use of a particular lacquering system which enables the drug to be administered more effectively.

It should be borne in mind that an essential feature of the administration of active ingredients is that they should have a palatable taste since they are released during the progressive chewing of the gum.

For drugs which are bitter or have little taste but nevertheless have very rapid release kinetics, tests have therefore also been carried out on coating them with the use of the microencapsulation technique; according to the particular kinetic results to be achieved, sometimes, the microencapsulation technique was not used on the whole of the active ingredient under investigation but only on some of it in order to keep a proportion for immediate action and the rest for delayed action.

By way of non-limiting example, this technology is effective for drugs such as dimenhydrinate, the effect of which against nausea generally needs to be developed rapidly for a certain proportion and then to continue for as long as possible in the bloodstream. The same result is sought in analgesic, antipyretic, cough-suppressant and antihistamine drugs, etc.

This expedient also sometimes entirely eliminates the side effects of some active ingredients on palatability, the use of this technology simultaneously achieving two quite separate objects.

Chewing gum preparations are particularly acceptable to children who can ingest drugs with a pleasant taste with the use of a more congenial form of ingestion closer to a normal sweet.

Moreover, for active ingredients which are easily oxidisable, degradable or hygroscopic, certain coatings are used to stabilize them during the steps of the process to which they are subjected, ensuring that they are preserved better over time.

The present invention achieves the objects set with the use of two distinct features, that is, the use of active ingredients as they are, as microencapsulated powders, or coated, mixed with one another in various ratios, and the lacquering of the finished pharmaceutical form.

The technology used for the preparation of the gums indicated is described in broad terms, by way of non-limiting example, below.

STEP 1

The gum is sold in pellets which, in order to be easily workable and thus to be mixed with other components, are frozen to a temperature of between −20° C. and −25° C. in a suitable chamber.

This step enables the gum to be processed without problems like any raw chemical product presented as a non-homogeneous powder.

In fact frozen gum is easily ground with a Danioni mill to produce a fairly homogeneous granulate generally with a particle size of between 190 and 60 mesh.

The granulate thus obtained:

1) can be mixed easily with the bases used as sweeteners in a suitable 4-way rotary-blade or screw mixer in proportions of ⅓ of gum and ⅔ of sweet base up to ⅘ of gum and ⅕ of sweet base; the sweet base is produced with sugars such as dextrose, glucose, sucrose, invert sugar, fructose, mannose, or maltose, or with polyalcohols used as sweeteners such as sorbitol, maltitol, xylitol or marmitol, or with synthetic sweeteners such as saccharine, acesulfame or aspartame, as well as with mixtures of any of the sweeteners mentioned above in various proportions to produce a palatable finished product with an acceptable taste;

2) after it has been mixed with the sweetening components, it can be granulated moist and dried on a fluid bed.

STEP 2

The mixture obtained in point 1) or the granulate obtained in point 2) is supplemented with a lubricant such as Na or Mg/Ca stearate in a proportion generally of between 0.2% and 2%, or with stearic acid or hydrogenated vegetable oils or other lubricants permitted by the pharmaceutical regulations (such as hydrogenated castor oil or palm butter). For some preparations, it is sometimes also appropriate to use additives such as microgranular cellulose in quantities of between 0.1 and 2% and between 0.05 and 1% of precipitated silica.

The mixture as produced above can proceed to the flavouring stage with the use of flavourings in either liquid or powder form.

After the addition of the active ingredient or ingredients, as they are or wholly or partially coated, the whole mixture is then compressed with a rotary press provided with suitable punches which should be polished, chrome-plated or Teflon-coated.

The tablets thus produced are ready to be film-coated as if they were normal tablets containing active ingredients. The gum tablets are placed in a heated vessel with blown hot air, with spraying equipment, and with forced extraction.

The gum tablets are thus spray-lacquered with the use of lacquers usually prepared with suitable mixtures based on hydroxypropylmethyl cellulose, polyethylene glycol 6000, polyethylene glycol 400 and pigments, all dispersed in demineralized water or in solvents formed by alcohol/water or acetone/alcohol/water mixtures. The gum tablets, which are put back in a vessel, are lacquered with lacquers thus formed, at a working temperature which may vary between 30° C. and 40° C.

Alternatively, alcoholic, aqueous-alcoholic or acetonic shellac lacquers of other cellulose derivatives such as hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetophthalate, carboxymethyl cellulose, hydroxyethyl cellulose, methylhydroxyethyl cellulose, or methylhydroxypropyl cellulose phthalate may be used for coating the gum tablets.

The gums in tablet form are then polished with carnauba wax and packed in suitable "blister" packs.

The technology described above produces finished products of palatable drugs such as vitamins and antihistamines, anti-inflammatories, dental products, products for the treatment of the main respiratory tracts, etc.

Stability tests have shown the drugs used to be very stable both from the point of view of protection against physical agents such as oxygen and moisture and as regards resistance to the effects of heat and light.

For some drugs, optimal kinetic curves have been obtained precisely with the use of this technology and with the use of a portion of the normal product combined with a portion of the microencapsulated product. Some examples of gum compositions are given purely by way of example:

1. VITAMIN C - 1.5 g of gum containing 250 mg of Vitamin C.

Gum base	0.800 g
Sorbitol	0.400 g
Vitamin C, 98% coated	0.250 g
Aspartame	0.010 g
Flavourings	0.015 g
Magnesium stearate	0.015 g
Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

2. TRICLOSAN 1.4 g of gum containing 0.010 mg of Triclosan

Gum base	0.850
Sorbitol	0.410 g
Triclosan	0.00001 g
Aspartame	0.09999 g
Flavourings	0.015 g
Magnesium stearate	0.015 g
Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

3. CETYL PYRIDINIUM - 1.5 g of gum containing 1 mg of cetyl pyridinium

Gum base	0.950 g
Sorbitol	0.500 g
Cetyl pyridinium	0.001 g
Aspartame	0.010 g
Flavourings	0.014 g
Magnesium stearate	0.015 g
Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

4. DIMENHYDRINATE - 1.5 g of gum containing 25 mg of dimenhydrinate

Gum base	0.950 g
Sorbitol	0.475 g
Dimenhydrinate, 50% microspheres	0.036 g
Dimenhydrinate, normal	0.007 g
Aspartame	0.010 g
Flavourings	0.015 g
Magnesium stearate	0.015 g
Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

5. CAMOMILE - 1.5 g of gum containing 250 mg of extract of camomile.

Gum base	0.800 g
Sorbitol	0.415 g
Camomile extract	0.250 g
Aspartame	0.010 g
Magnesium stearate	0.015 g
Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

6. ASPIRIN - 1.5 g of gum containing 300 mg of aspirin

Gum base	0.750 g
Sorbitol	0.400 g
Aspirin	0.300 g
Aspartame	0.010 g
Flavourings	0.015 g
Magnesium stearate	0.015 g

-continued

Hydroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

7. B-CAROTENE + VITAMIN E - 1.5 g of gum containing 25 mg of vitamin E and 50 mg of B-carotene

Gum base	0.850 g
Sorbitol	0.500 g
Vitamin E, 50% coated	0.050 g
β-carotene	0.050 g
Aspartame	0.010 g
Flavourings	0.015 g
Magnesium stearate	0.015 g
Hyroxypropylmethyl cellulose	0.008 g
Colourings	0.002 g
Distilled water	0.090 g

For slightly soluble active ingredients which nevertheless have moderate palatability, gum tablets which release the active ingredient immediately have been produced according to the formulations given below.

8. MEBENDAZOLE - 1.24 g gum tablet containing 200 mg of mebendazole

Gum base	474.8 mg
Sorbitol	462.2 mg
Hydroxypropylmethyl cellulose	23 mg
Glycerol	21.6 mg
Menthol	18.2 mg
Magnesium stearate	12 mg
Aspartame	8 mg
Essential oils of mint	8 mg
Polyethylene glycol	7 mg
Titanium dioxide	5 mg
Quinoline yellow colouring	0.2 mg

9. SUCRALFATE - 1.5 g gum tablet containing 250 mg of sucralfate and possibly 100 mg of calcium carbonate

Sorbitol	587.600 mg
Gum base	450 mg
Essential oil of mint	24 mg
Menthol	21 mg
Glycerol	20 mg
Hydroxypropylmethyl cellulose	12.875 mg
Magnesium stearate	12 mg
Titanium dioxide	12 mg
Polyethylene glycol	5 mg
Aspartame	4.400 mg
Anethole	1 mg
Chlorophyll green	0.125 mg

In the case of unpalatable active ingredients such as Benzydamine (3 mg), Cimetidine (100 mg), Ibuprofen (200 mg), Nimesulide (50 mg), etc., sugary microgranules are prepared and the various active ingredients subsequently to be mixed with the chewing gum are adsorbed thereon.

These microgranules are then coated with the usual excipients and are then mixed with the gums. The technology used and some examples of the application thereof are given by way of non-limiting information:

A. Sugary microgranules of 850 microns diameter were introduced into a vessel provided with automatic spraying equipment and a system for blowing in hot air at 40°/80° C. and for recovering the blown air. If the formula requires it, the granules may be moistened with suitable flavouring essences before enlargement with syrup.

B. A syrup, possibly suitably flavoured, containing the micronized drug in suspension, was prepared (the mean quantities of drug which can be dispersed vary between 1 and 15% by weight of the syrup).

C. The granules were enlarged, care being taken to sift them frequently to prevent lumps and accumulations.

D. When all of the syrup had been absorbed by the granules, they were weighed to check how much of the drug had actually been absorbed, in order to determine the theoretical strength.

E. After the vessel had been carefully washed, the finished microgranules were coated therein with a solution of hydroxypropylmethyl cellulose in alcohol or with other lacquers suitable for rendering the granules Bore or less gastro-resistant, for example, lacquers based on methyl cellulose, acetyl cellulose, cellulose acetophthalate, etc.

10. BENZYDAMINE - gum tablets containing 3.0 mg of benzimidamine hydrochloride

Lemery gum	233.0 mg
Nostic gum	233.0 mg
Sorbitol	593.0 mg
Menthol	17.0 mg
Essential oil of peppermint L	7.35 mg
Glycerol	21.0 mg
Aspartame	4.3 mg
Sucrose	243.0 mg
Starch	91.0 mg
Magnesium stearate	12.0 mg
Precipitated silica	5.0 mg
Mint flavouring	6.0 mg
Lemon flavouring	7.0 mg
Anethole	1.0 mg
Peppermint	5.0 mg
Sweet mint	3.0 mg

11. NIMESULIDE - A 1.55 g gum tablet containing 50 mg of Nimesulide

Sorbitol	458 mg
Gum base	439 mg
Sucrose	260 mg
Sugary microspheres 850 mu	180 mg
Orange flavouring	55.6 mg
Citric acid	27 mg
Hydroxypropylmethyl cellulose	23 mg
Glycerol	18 mg
Rice starch	10 mg
Magnesium stearate	10 mg
Polyethylene glycol	7 mg
Aspartame	5.2 mg
Titanium dioxide	5 mg
Yellow colouring E102	2 mg
Red colouring E124	0.2 mg

All of these examples should be considered purely as non-limiting examples, since the technology described can be applied without distinction to all pharmaceutically active ingredients with good absorption results. In fact the technology of the present invention enables the active ingredient to be modulated better and more conveniently with the use of the coating method made available by the pharmaceutical prior art for microencapsulation.

The fact that it is possible to regulate the mixing ratio between the active ingredient for immediate release and the active ingredient for slow release, combined with the particular lacquering system, simultaneously satisfies and reconciles several requirements, that is, taste and palatability, compliance by the patient and improved plasmatic and haematic absorption of the drug.

We claim:

1. Chewing gum tablet comprising:

a mixture of a chewing gum base and sugary microgranules;

a component adsorbed onto said sugary microgranules selected from the group consisting of an additive agent and an active ingredient; and

a lacquer coating on the tablet selected from the group consisting of pharmaceutically acceptable celluloses and polyethylene glycols.

2. Chewing gum tablet according to claim 1, wherein said additive agent is a flavoring agent.

3. Chewing gum tablet according to claim 1, wherein said at least one active ingredient is in form of microencapsulated or otherwise delayed release coated particles.

4. Chewing gum tablet according to claim 1, wherein the cellulose is selected are selected from the group consisting of hydroxypropylmethyl cellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetophthalate, carboxymethyl cellulose, hydroxyethyl cellulose, methylhydroxyethyl cellulose and methylhydroxypropyl cellulose phthalate.

5. Chewing gum tablet according to claim 1, wherein the polyethylene glycol is selected from the group consisting of polyethylene glycol 6000 and polyethylene glycol 400.

6. Chewing gum tablet according to claim 1, wherein the lacquer is supplemented with an additional component selected from the group consisting of colorings and dyes.

7. Chewing gum tablet according to claim 2, wherein some said particles of said active ingredient are microencapsulated and some are in free form in a predetermined ratio with regard to the patterns of initial delivery and of maintenance of the blood levels.

8. A method of preparing a tablet, comprising the steps of:

a) freezing chewing gum in pellet form to a temperature of between −20° C. and −25° C. to form frozen gum;

b) grinding said frozen gum to a particle size of between 60 and 190 mesh to form ground chewing gum;

c) adding to said ground chewing gum sugary microgranules having adsorbed onto a surface thereof a component selected from the group consisting of an additive agent and au active ingredient to form a granular mixture;

d) compressing said granular mixture to form tablets; and

e) coating said tablets with a lacquer comprising a pharmaceutically acceptable celluloses or a polyethylene glycol in a solvent.

9. A method according to claim 8, wherein said ground chewing gum is mixed with at least one natural or synthetic sweetener in a ratio of 0.3–0.8 parts of gum per 0.6–0.2 parts of sweetener phase.

10. A method according to claim 9, wherein said additive agent is selected from the group consisting of a lubricant and a flavoring agent.

11. A method according to claim 10, wherein said active ingredient is added to the mixture of ground chewing gum, sweetener, lubricant and flavoring agent in the form of microencapsulated or otherwise delayed release coated particles.

12. A method according to claim 8, wherein said solvent is selected from the group consisting of water, an alcohol, acetone, and mixtures thereof.

13. A method according to claim 9, wherein said sweetener is selected from the group consisting of sugars, polyalcohols used as sweeteners, saccharin, acesulfame, aspartame and mixtures thereof.

14. A method according to claim 13, wherein the sugar is selected from the group consisting of dextrose, glucose, sucrose, invert sugar, fructose, mannose and maltose.

15. A method according to claim 13, wherein the polyalcohols are selected from the group consisting of sorbitol, mannitol, maltitol and xylitol.

16. A method according to claim 9, wherein the mixture of gum and sweetener is granulated moist and is dried on a fluid bed.

17. A method according to claim 8, wherein the mixture of said frozen chewing gum pellets and said sugary microgranules is granulated in moistened condition and dried on a fluid bed and then tablets are prepared by compression therefrom.

18. A method according to claim 10, wherein the lubricant is selected from the group consisting of alkali-metal or alkaline-earth metal stearates, stearic acid, hydrogenated vegetable oils and other lubricants used in the preparation of tablets for pharmaceutical use, and is added in an mount of between 0.2% and 2% by weight relative to the weight of the composition.

19. A method according to claim 10, wherein microgranular cellulose and/or precipitated silica are added together with said lubricant.

20. A method according to claim 19, wherein the microgranular cellulose is added in an amount of between 0.1% and 2% by weight.

21. A method according to claim 19, wherein the precipitated silica is added in quantities of between 0.05% and 1% by weight.

22. A method according to claim 8, wherein the flavoring agent is in liquid or powder form.

23. A method according to claim 8, wherein the lacquer is sprayed in a heated vessel with hot air.

24. A chewing gum composition comprising:

a mixture of a chewing gum base and sugary microgranules;

a component adsorbed onto said sugary microgranules selected from the group consisting of an additive agent and an active ingredient; and

a lacquer coating on said microgranules selected from the group consisting of pharmaceutically acceptable celluloses and polyethylene glycols.

25. Chewing gum composition according to claim 24, wherein said additive agent is a flavoring agent.

26. Chewing gum composition according to claim 24, wherein said at least one active ingredient is in form of microencapsulated or otherwise delayed release coated particles.

27. Chewing gum composition according to claim 24, wherein the cellulose is selected are selected from the group consisting of hydroxypropylmethyl cellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetophthalate, carboxymethyl cellulose, hydroxyethyl cellulose, methylhydroxyethyl cellulose and methylhydroxypropyl cellulose phthalate.

28. Chewing gum composition according to claim 24, wherein the polyethylene glycol is selected from the group consisting of polyethylene glycol 6000 and polyethylene glycol 400.

29. Chewing gum composition according to claim 24, wherein the lacquer is supplemented with an additional component selected from the group consisting of colorings and dyes.

30. Chewing gum tablet according to claim 26, wherein some said particles of said active ingredient are microencapsulated and some are in free form in a predetermined ratio with regard to the patterns of initial delivery and of maintenance of the blood levels.

31. A method of preparing a chewing gum composition, comprising the steps of:

a) providing sugary microgranules having adsorbed onto a surface thereof a component selected from the group consisting of an additive agent and an active ingredient;

b) coating said sugary microgranules with a lacquer comprising a pharmaceutically acceptable celluloses or a polyethylene glycol in a solvent to form coated microgranules;

c) mixing said coated microgranules with frozen ground chewing gum to form a chewing gum composition.

32. A method according to claim 31, wherein said chewing gum is frozen in pellet form to a temperature of between −20° C. and −25° C. and ground to a particle size of between 60 and 190 mesh.

33. A method according to claim 31, wherein said ground chewing gum is mixed with at least one natural or synthetic sweetener in a ratio of 0.3–0.8 parts of gum per 0.6–0.2 parts of sweetener phase.

34. A method according to claim 31, wherein said additive agent is selected from the group consisting of a lubricant and a flavoring agent.

35. A method according to claim 31, wherein said active ingredient is in the form of microencapsulated or otherwise delayed release coated particles.

36. A method according to claim 31, wherein said solvent is selected from the group consisting of water, an alcohol, acetone, and mixtures thereof.

37. A method according to claim 33, wherein said sweetener is selected from the group consisting of sugars, polyalcohols used as sweeteners, saccharin, acesulfame, aspartame and mixtures thereof.

38. A method according to claim 37, wherein the sugar is selected from the group consisting of dextrose, glucose, sucrose, invert sugar, fructose, mannose and maltose.

39. A method according to claim 37, wherein the polyalcohols are selected from the group consisting of sorbitol, mannitol, maltitol and xylitol.

40. A method according to claim 34, wherein the lubricant is selected from the group consisting of alkali-metal or alkaline-earth metal stearates, stearic acid, hydrogenated vegetable oils and other lubricants used in the preparation of tablets for pharmaceutical use, and is added in an mount of between 0.2% and 2% by weight relative to the weight of the composition.

41. A method according to claim 34, wherein the flavoring agent is in liquid or powder form.

42. A method according to claim 31, wherein the lacquer is sprayed in a heated vessel with hot air.

43. A method according to claim 31 and further including the step of compressing the chewing gum composition to form a tablet.

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2. U.S. Patent No. 6,375,986 to Ryde et al.

US006375986B1

(12) **United States Patent**
Ryde et al.

(10) **Patent No.: US 6,375,986 B1**
(45) **Date of Patent: Apr. 23, 2002**

(54) **SOLID DOSE NANOPARTICULATE COMPOSITIONS COMPRISING A SYNERGISTIC COMBINATION OF A POLYMERIC SURFACE STABILIZER AND DIOCTYL SODIUM SULFOSUCCINATE**

(75) Inventors: **Niels P. Ryde**, Malvern; **Stephen B. Ruddy**, Schwenksville, both of PA (US)

(73) Assignee: **Elan Pharma International Ltd.**, Clare (IE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(58) **Field of Search** 424/489, 466, 424/464, 434, 44, 43; 514/535

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5,543,133	A	8/1996	Swanson et al.	424/9.45
5,552,160	A	9/1996	Liversidge et al.	424/489
5,560,931	A	10/1996	Eichoff et al.	424/489
5,560,932	A	10/1996	Bagchi et al.	424/489
5,565,188	A	10/1996	Wong et al.	424/9.411
5,571,536	A	11/1996	Eickhoff et al.	424/489
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WO	96/25918	8/1996
WO	98/35666	8/1998
WO	00/18374	4/2000
WO	00/27363	5/2000

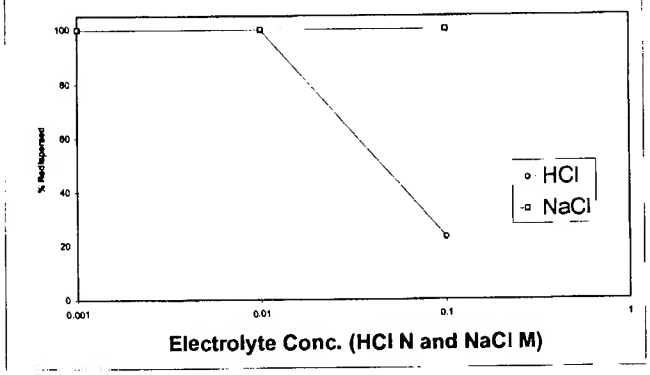
Primary Examiner—Diana Dudash
Assistant Examiner—Mina Haghighation
(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) **ABSTRACT**

Disclosed are solid dose nanoparticulate compositions comprising a poorly soluble active agent, at least one polymeric surface stabilizer, and dioctyl sodium sulfosuccinate (DOSS). The solid dose compositions exhibit superior redispersibility of the nanoparticulate composition upon administration to a mammal, such as a human or animal. The invention also describes methods of making and using such compositions.

34 Claims, 1 Drawing Sheet

FIGURE 1: Plasdone S630 + DOSS



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5,587,143	A	12/1996	Wong	424/9.1
5,593,657	A	1/1997	Ruddy et al.	424/9.41
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5,643,552	A	7/1997	Illig	424/9.45
5,665,330	A	9/1997	Wong	424/9.44
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5,834,025	A	11/1998	De Garavilla et al.	424/501
6,045,829	A	4/2000	Liversidge et al.	424/489
6,068,858	A	5/2000	Liversidge et al.	424/489
6,165,506	A	* 12/2000	Jain et al.	424/466

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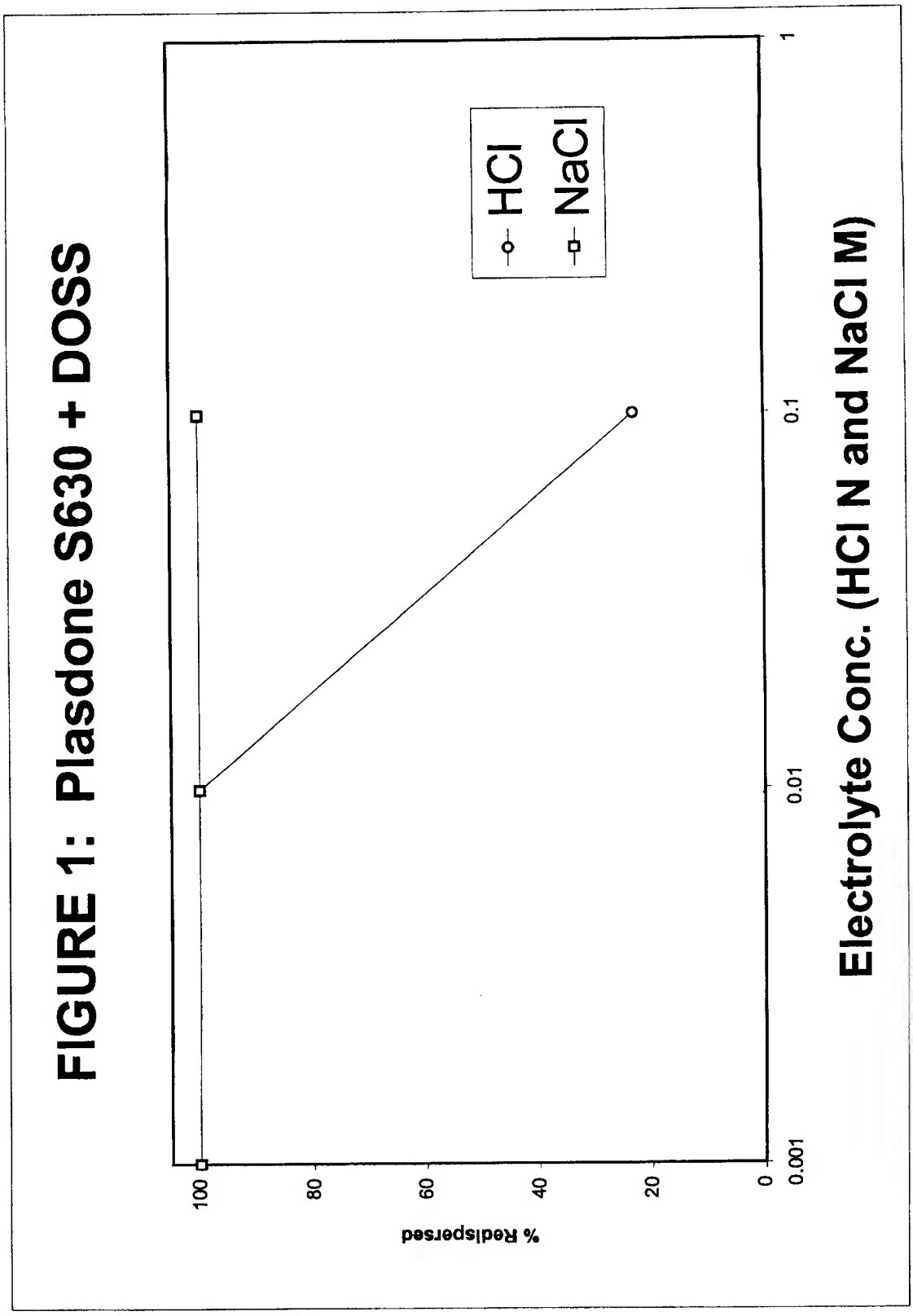


FIGURE 1: Plasdone S630 + DOSS
HCl
NaCl
% Redispersed
100
80
60
40
20
0
0.001
0.01
0.1
1
Electrolyte Conc. (HCl N and NaCl M)

SOLID DOSE NANOPARTICULATE COMPOSITIONS COMPRISING A SYNERGISTIC COMBINATION OF A POLYMERIC SURFACE STABILIZER AND DIOCTYL SODIUM SULFOSUCCINATE

FIELD OF THE INVENTION

The present invention is directed to solid dose nanoparticulate compositions having a synergistic combination of at least one polymeric surface stabilizer and dioctyl sodium sulfosuccinate (DOSS). The solid dose compositions exhibit superior redispersion of the nanoparticulate composition either upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution.

BACKGROUND OF THE INVENTION

A. Background Regarding Nanoparticulate Compositions

Nanoparticulate compositions, first described in U.S. Pat. No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble therapeutic or diagnostic agent having adsorbed onto the surface thereof a non-crosslinked surface stabilizer. This invention is an improvement over that disclosed in the '684 patent, as the '684 patent does not describe the use of synergistic combinations of polymeric surface stabilizers and DOSS in solid dose compositions.

Prior U.S. patents teach the use of DOSS as a primary or secondary surface stabilizer for nanoparticulate compositions. See e.g., U.S. Pat. No. 5,145,684, for "Surface Modified Drug Nanoparticles;" U.S. Pat. No. 5,302,401, for "Method to Reduce Particle Size Growth During Lyophilization;" U.S. Pat. No. 5,318,767, for "X-Ray Contrast Compositions Useful in Medical Imaging;" U.S. Pat. No. 5,336,507, for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" U.S. Pat. No. 5,346,702, for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" U.S. Pat. No. 5,399,363, for "Surface Modified Anticancer Nanoparticles;" U.S. Pat. No. 5,401,492, for "Water-Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" U.S. Pat. No. 5,429,824, for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" U.S. Pat. No. 5,451,393, for "X-Ray Contrast Compositions Useful in Medical Imaging;" U.S. Pat. No. 5,466,440, for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" U.S. Pat. No. 5,470,583, for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" U.S. Pat. No. 5,494,683, for "Surface Modified Anticancer Nanoparticles;" U.S. Pat. No. 5,503,723, for "Isolation of Ultra Small Particles;" U.S. Pat. No. 5,510,118, for "Process for Preparing Therapeutic Compositions Containing Nanoparticles;" U.S. Pat. No. 5,543,133, for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Pat. No. 5,552,160, for "Surface Modified NSAID Nanoparticles;" U.S. Pat. No. 5,560,931, for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,560,932, for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Pat. No. 5,571,536, for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,580,579, for "Site-Specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" U.S. Pat. No. 5,587,143, for "Butylene Oxide-Ethylene Oxide Block Copolymer Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" U.S. Pat. No. 5,593,657, for "Novel Barium Salt Formulations Stabilized by Non-Ionic and Anionic Stabilizers;" U.S. Pat. No. 5,628,981, for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" U.S. Pat. No. 5,665,331, for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Pat. No. 5,716,642, for "Microprecipitation of Nanoparticulate Pharmaceutical Agents Using Surface Active Material Derived from Similar Pharmaceutical Agents;" U.S. Pat. No. 5,718,919, for "Nanoparticles Containing the R(−) Enantiomer of Ibuprofen;" U.S. Pat. No. 5,747,001, for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" U.S. Pat. No. 5,834,025, for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" U.S. Pat. No. 6,045,829, for "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" and U.S. Pat. No. 6,068,858, for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers." In addition, several published international applications teach the usefulness of DOSS as a primary or secondary surface stabilizer for nanoparticulate compositions. See e.g., WO 98/35666, for "Formulations of Nanoparticle Naproxen Tablets;" WO 00/18374, for "Controlled Release Nanoparticulate Compositions;" WO 96/25918, for "Aerosols Containing Nanoparticulate Dispersions;" and WO 00/27363, for "Aerosols Comprising Nanoparticle Drugs."

Prior art patents also teach the use of DOSS as a cloud point modifier for nanoparticulate surface stabilizers. See e.g., U.S. Pat. No. 5,298,262, for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. No. 5,326,552, for "Novel Formulation for Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weigh Non-ionic Surfactants;" U.S. Pat. No. 5,346,702, for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" U.S. Pat. No. 5,352,459, for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. No. 5,447,710, for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-Ionic Surfactants;" U.S. Pat. No. 5,565,188, for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" U.S. Pat. No. 5,665,330, for "Dual Purpose Diagnostic/Therapeutic Agent Having a Tri-Iodinated Benzoyl Group Linked to a Coumarin."

And several prior art references teach the use of DOSS in nanoparticulate compositions as both a surface stabilizer and as a cloud point modifier for a primary surface stabilizer. See e.g., U.S. Pat. No. 5,466,433, for "Polyiodinated Aroyloxy Esters;" U.S. Pat. No. 5,472,683, for "Nanoparticle Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,500,204, for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,521,218, for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" U.S. Pat. No. 5,525,328, for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic Systems Imaging;" U.S. Pat. No. 5,534,270, for "Method of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Pat. No. 5,573,749, for "Nanoparticulate Diagnostic Mixed Carboxylic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,573,750, for

"Diagnostic Imaging X-Ray Contrast Agents;" U.S. Pat. No. 5,603,916, for "3,5-Bis-[Alkanoyl Amino]-2,4,6-Triiodobenzyl Esters;" U.S. Pat. No. 5,643,552, for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,668,196, for "3-Amido-Triiodophenyl Esters as X-Ray Contrast Agents;" and U.S. Pat. No. 5,670,136, for "2,4,6-Triiodo-5-Substituted-Amino-Isophthalate Esters Useful as X-Ray Contrast Agents for Medical Diagnostic Imaging."

U.S. Pat. No 5,585,108, for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays," claims a nanoparticulate dispersion including, inter alia, a water-insoluble particulate drug, a surfactant which can be a polymeric stabilizer, such as hydroxypropyl methylcellulose, a pharmaceutically acceptable clay, and a secondary stabilizer, such as DOSS or sodium lauryl sulfate. See col. 7 of the patent. This reference differs from the present invention in that it is directed to a nanoparticulate dispersion, and not a solid dose nanoparticulate formulation.

U.S. Pat. No 5,298,262, for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization," describes the use of DOSS in a nanoparticulate composition as an anionic surfactant useful in raising the cloud point of a surface stabilizer. According to the '262 patent, by raising the cloud point of the surface stabilizer of a nanoparticulate composition, the composition can be heat sterilized without producing particle aggregation because of the exposure to elevated temperatures. Liquid compositions are heat sterilized, not powders. This is because sterile products are not manufactured for oral administration because of the cost, complexity, etc. Thus, this patent does not teach or suggest the use of DOSS in a solid dose formulation to increase redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution.

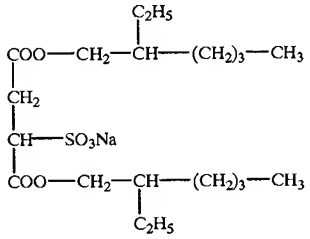
Finally, U.S. Pat. No. 5,518,738, for "Nanoparticulate NSAID Compositions," describes a nanoparticulate solid dose of an NSAID having a film of polyvinylpyrrolidone (PVP), hygroscopic sugar, and sodium lauryl sulfate adsorbed on the surface of the drug. In the examples of this patent, solid films of the nanoparticulate composition with various redispersants are prepared, including DOSS. In contrast to the present invention, the '738 patent teaches that a solid film of a nanoparticulate drug, DOSS, and PVP shows extremely poor redispersibility. Thus, this reference teaches away from the present invention.

Many of the prior art patents listed above also teach the usefulness of polymeric surface stabilizers for nanoparticulate compositions, such as hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and polyvinylpyrrolidone.

However, the prior art does not teach or suggest the use of synergistic combinations of polymeric surface stabilizers and DOSS in solid dose compositions of nanoparticulate active agents. Nor does the prior art teach or suggest that such synergistic compositions can result in superior redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution.

B. Background Regarding DOSS

DOSS is an anionic surfactant commercially available from a variety of sources, including Chemax Inc. (Greenville, S.C.), Finetex Inc. (Elmwood Park, N.J.), R. W. Greeff & Co. (Greenwich, Conn.), McIntyre Group Ltd. (Chicago, Ill.), Penta Mfg. Co. (Livingston, N.J.), Rhone-Poulenc Inc. Specialty Chemicals Div., (Cranbury, N.J.), RTD Chemicals Corp. (Hackettown, N.J.), Scher Chemicals Inc. (Clifton, N.J.), Spectrum Quality Products Inc. (Gardena, Calif.), Thornley Co. Inc. (Wilmington, Del.), and Van Waters & Rogers (Kirkland, Wash.). It has the chemical formula $C_{20}H_{37}O_7SNa$ and the following structure:



DOSS is a widely used wetting agent and dispersant. It is a white, waxlike, plastic solid added to powdered gelatins, drink mixes, and cocoas to make them dissolve more quickly and completely in liquids. It is also used as a stabilizer in pharmaceuticals, chewing gums, and canned milks, and is added to shampoos, bath products, and skin cleansers. While the U.S. Food and Drug Administration (FDA) limits the amount of DOSS that can be used in food and drug products, it still rates the compound generally recognized as safe (GRAS). 21 C.F.R. § 172.810.

There is a need in the art for solid dose nanoparticulate compositions exhibiting superior redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to the surprising and unexpected discovery that solid dose nanoparticulate compositions comprising at least one polymeric surface stabilizer and DOSS exhibit superior redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution. The solid dose nanoparticulate compositions comprise at least one poorly soluble active agent, at least one polymeric surface stabilizer adsorbed to the surface of the active agent, and DOSS.

Another aspect of the invention is directed to pharmaceutical compositions comprising a solid dose nanoparticulate composition of the invention. The pharmaceutical composition comprises at least one poorly soluble active agent, at least one polymeric surface stabilizer adsorbed to the surface of the drug, DOSS, and a pharmaceutically acceptable carrier, as well as any desired excipients.

This invention further discloses methods of making a nanoparticulate composition having at least one polymeric surface stabilizer adsorbed on the surface of the active agent and DOSS. Such a method comprises contacting a poorly soluble nanoparticulate active agent with at least one polymeric surface stabilizer and DOSS under time and conditions sufficient to provide a nanoparticle active agent/surface stabilizer/DOSS composition. Some or all of the polymeric surface stabilizers and DOSS can be contacted with the active agent either before, during, or after size reduction of the active agent.

The present invention is further directed to methods of treatment comprising administering to a mammal in need a therapeutically effective amount of a nanoparticulate composition according to the invention.

Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Shows the % redispersion in an electrolyte solution, as a function of the concentration of the electrolyte solution, for a spray dried nanoparticulate MAP kinase inhibitor composition.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the surprising and unexpected discovery that solid dose nanoparticulate compositions having at least one polymeric surface stabilizer and DOSS exhibit dramatically superior redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or upon reconstitution of a dry powder prepared from a nanoparticulate composition in an aqueous electrolyte solution. The electrolyte concentration should be representative of physiological conditions found in the human body. Representative electrolyte solutions can be, but are not limited to, 0.1, 0.01, or 0.001 N HCL, and/or 0.1, 0.01, or 0.001 M NaCl, and combinations thereof. Of these electrolyte solutions, 0.01 N HCl, 0.1 M NaCl, and combinations thereof are most representative of human physiological conditions.

Prior to the present invention, liquid dispersions and solid dose forms of nanoparticulate compositions were known. One frequent problem of prior art solid dose nanoparticulate compositions was that upon administration to a mammal, such as a human or animal, the nanoparticulate composition would not redisperse, and thus the solid dose composition would lose the benefits afforded by formulating the composition into a nanoparticulate form. This is because nanoparticulate compositions benefit from the small particle size of the active agent; if the active agent does not redisperse into the small particle sizes upon administration, then "clumps" or agglomerated drug particles are formed. With the formation of such agglomerated particles, the bioavailability of the composition drops dramatically below that observed with the liquid dispersion form of the drug.

Most drugs are marketed in a solid dose form, such as a tablet, capsule, etc. This is because such dosage forms are easy to store and transport. In addition, such dosage forms are easily marketed. Patient compliance is high, as compared with injectable forms of drugs. Thus, it is critical to develop solid dose forms of nanoparticulate compositions which exhibit the same benefits observed with the liquid dispersion form of the compositions.

It was discovered that solid dose nanoparticulate compositions having at least one polymeric surface stabilizer and DOSS exhibit dramatic redispersion of the nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution. DOSS or polymeric stabilizers alone cannot produce highly redispersible solid dose nanoparticulate compositions. In combination, however, the two compounds exhibit a synergistic effect of stabilizing the active agent and resulting in dramatic redispersion of the solid dose nanoparticulate composition upon administration to a mammal, such as a human or animal, or reconstitution in an aqueous electrolyte solution.

Another benefit of the invention is that DOSS is highly tolerated by the human body, in contrast to other dispersants such as SLS, for which the human body has a low tolerance. DOSS can be given to humans in large doses on a chronic basis, as the FDA has approved the use of DOSS as a stool softener at doses of up to 500 mg/daily for adults, and in children over 6 months old up to 75 mg/day. See *Handbook of Pharmaceutical Excipients,* Third Edition, p. 189 (American Pharmaceutical Association, 2000). The dosage of DOSS employed in the present invention is below the threshold amount which produces laxative effects.

The combination of DOSS and a polymeric surface stabilizer was tested on a wide variety of drugs, including Mitogen-Activated protein (MAP) kinase inhibitor, an analgesic, and an angiogenesis inhibitor. Thus, the phenomenon of high redispersibility is not limited to a specific drug or drug class. However, the phenomenon is limited to nanoparticulate compositions comprising at least one polymeric surface stabilizer and DOSS. Other types of surface stabilizers formulated with DOSS, such as ampiphilic stabilizers having hydrophobic and hydrophilic ends, have not been found to produce solid dose compositions having comparable redispersion properties.

A. Nanoparticulate Compositions

The nanoparticulate compositions of the invention comprise a nanoparticulate active agent, such as a drug, having at least one polymeric surface stabilizer adsorbed on the surface thereof and DOSS. The nanoparticulate active agent compositions, comprising a nanoparticulate active agent and at least one polymeric surfactant, have an effective average particle size prior to incorporation in a solid dose form of less than about 1 micron, less than about 800 nm, less than about 600 nm, less than about 400 nm, and less than about 200 nm.

Upon administration to a mammal, such as a human or animal, or reconstitution in an electrolyte solution, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about (1) 5 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 1 micron; (2) 4 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 800 nm; (3) 3 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 600 nm; (4) 2 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 400 nm; and (5) 1 micron, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 200 nm.

1. Drug Particles

The nanoparticles of the invention comprise a therapeutic or diagnostic agent, collectively referred to as a "drug," which is poorly soluble in at least one medium. By "poorly soluble" it is meant that the drug has a solubility in the liquid dispersion medium of less than about 10 mg/mL, and preferably of less than about 1 mg/mL. A therapeutic agent can be a pharmaceutical agent, including biologics such as proteins, peptides, and nucleotides, or a diagnostic agent, such as a contrast agent, including x-ray contrast agents. The drug is preferably present in an essentially pure form, is dispersible in at least one liquid medium, and exists either as a discrete, crystalline phase, or as an amorphous phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as those described in EP Patent No. 275,796.

The drug can be selected from a variety of known classes of drugs, including, for example, proteins, peptides, nucleotides, anti-obesity drugs, nutriceuticals, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, anti-muscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radiopharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines.

The drugs are commercially available and/or can be prepared by techniques known in the art.

2. Surface Stabilizers

Polymeric surface stabilizers useful herein physically adhere to the surface of the nanoparticulate active agent, but do not chemically react with the drug or itself. Individually adsorbed molecules of the surface stabilizer are essentially free of intermolecular cross-linkages.

The polymeric surface stabilizer is adsorbed on the surface of the active agent in an amount sufficient to maintain an effective average particle size of less than about 1 micron. Two or more surface stabilizers can be employed in the compositions and methods of the invention.

Representative examples of suitable polymeric surface stabilizers include, but are not limited to polyvinylpyrrolidone (PVP), cellulose ethers such as, but not limited to, hydroxypropyl cellulose, hydroxypropyl methylcellulose, carboxymethyl cellulose, methyl cellulose, and hydroxyethyl cellulose, polysaccharides such as, but not limited to, dextrin, guar gum, starch, random copolymers of vinyl acetate and vinyl pyrrolidone, such as Plasdone® S630 (ISP), Kollidone® VA 64 (BASF), polyvinyl alcohol, copolymers of vinylacetate and vinylalcohol.

Plasdone® S630 is a random copolymer of vinyl pyrrolidone and vinyl acetate, in a 60:40 ratio. Other random copolymers of vinyl pyrrolidone and vinyl acetate can also be used in the invention having, for example, ratios of vinyl pyrrolidone to vinyl acetate of 90:10, 80:20, or 50:50. Preferably, the random copolymer contains at least 50% vinyl pyrrolidone.

The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

3. Nanoparticulate Dru/Surface Stabilizer Particle Size

As used herein, particle size is determined on the basis of the weight average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, dynamic and static light scattering, and disk centrifugation.

By "an effective average particle size of less than about 1 micron" it is meant that at least 90% of the active agent particles have a particle size of less than about 1 micron when measured by the above techniques. In other embodiments, the nanoparticulate active agent compositions, comprising a nanoparticulate active agent and at least one polymeric surfactant, have an effective average particle size prior to incorporation in a solid dose form of less than about 800 nm, less than about 600 nm, less than about 400 nm, and less than about 200 nm.

Upon administration to a mammal, such as a human or animal, or reconstitution in an electrolyte solution, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about (1) 5 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 1 micron; (2) 4 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 800 nm; (3) 3 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 600 nm; (4) 2 microns, when the nanoparticulate dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 400 nm; and (5) 1 micron, when the nanocrystal dispersion, prior to incorporation into a solid dose form, has an effective average particle size of less than about 200 nm.

4. Other Pharmaceutical Excipients

Pharmaceutical compositions according to the invention may also comprise one or more binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients. Such excipients are known in the art.

Examples of filling agents are lactose monohydrate, lactose anhydrous, and various starches; examples of binding agents are various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, microcrystalline cellulose, and silicified microcrystalline cellulose (SMCC).

Suitable lubricants, including agents that act on the flowability of the powder to be compressed, are colloidal silicon dioxide, such as Aerosil® 200, talc, stearic acid, magnesium stearate, calcium stearate, and silica gel.

Examples of sweeteners are any natural or artificial sweetener, such as sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and accsulfame K. Examples of flavoring agents are Magnasweet® (trademark of MAFCO), bubble gum flavor, and fruit flavors, and the like.

Examples of preservatives are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quarternary compounds such as benzalkonium chloride.

Suitable diluents include pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing. Examples of diluents include microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhydrous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose.

Suitable disintegrants include corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, crosspovidone, sodium starch glycolate, and mixtures thereof.

Examples of effervescent agents are effervescent couples such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric,

malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the acid component of the effervescent couple may be present.

5. Concentration of Nanoparticulate Drug, Surface Stabilizer, and DOSS

The relative amount of drug, one or more polymeric surface stabilizers, and DOSS can vary widely. The optimal amount of the polymeric surface stabilizers can depend, for example, upon the particular drug selected, the equivalent hydrophilic lipophilic balance (HLB) of the drug, the melting point, cloud point, and water solubility of the polymeric surface stabilizer, and the surface tension of water solutions of the stabilizer, etc.

The concentration of the one or more polymeric surface stabilizers can vary from about 0.01 to about 90%, from about 1 to about 75%, from about 10 to about 60%, or from about 10 to about 55% by weight based on the total combined dry weight of the drug substance and surface stabilizer, not including other excipients.

The concentration of the drug can vary from about 99.8% to about 0.1%, from about 80% to about 5.0%, or from about 50% to about 10% by weight based on the total combined dry weight of the drug and polymeric surface stabilizer, not including other excipients.

The concentration of DOSS can vary from about 0.1 to about 20%, and from about 1 to about 10%, based on the total dry weight of the drug, surface stabilizer, and DOSS, not including other excipients.

B. Methods of Making Nanoparticulate Formulations

The nanoparticulate drug compositions can be made using, for example, milling or precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in the '684 patent.

1. Milling to obtain Nanoparticulate Drug Dispersions

Milling of aqueous drug dispersions to obtain a nanoparticulate dispersion comprises dispersing poorly soluble drug particles in a liquid dispersion medium, followed by applying mechanical means in the presence of grinding media to reduce the particle size of the drug to the desired effective average particle size. The drug particles can be reduced in size in the presence of at least one polymeric surface stabilizer and/or DOSS. Alternatively, the drug particles may be contacted with one or more polymeric surface stabilizers and/or DOSS after attrition. Other compounds, such as a diluent, can be added to the drug/surface stabilizer composition during the size reduction process. Dispersions can be manufactured continuously or in a batch mode. The resultant nanoparticulate drug dispersion can be utilized in solid dosage formulations, such as controlled release dosage formulations, solid dose fast melt formulations, aerosol formulations, tablets, capsules, etc.

2. Precipitation to Obtain Nanoparticulate Drug Compositions

Another method of forming the desired nanoparticulate composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble drugs in the presence of one or more polymeric surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving the poorly water-soluble drug in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one polymeric surface stabilizer and DOSS to form a solution; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means. The resultant nanoparticulate drug dispersion can be dried and used in a solid dose composition.

3. Methods of Drying Nanoparticulate Dispersions

The nanoparticulate liquid dispersion formed by either milling or precipitation can be dried prior to formulating the composition into a solid dose form for administration.

Powders comprising nanoparticulate drug can be made by spray-drying aqueous dispersions of a nanoparticulate drug, polymeric surface stabilizer, and DOSS to form a dry powder which consists of aggregated drug/polymeric surface stabilizer/DOSS nanoparticles. Alternatively, the aqueous dispersion of drug, polymeric surface stabilizer, and DOSS can contain a dissolved diluent, such as lactose or mannitol, which when spray dried forms diluent particles, each of which contains at least one embedded drug nanoparticle combined with a polymeric surface stabilizer and DOSS.

Nanoparticulate drug dispersions can also be freeze-dried to obtain powders suitable for formulation into solid dose forms. Such powders comprise aggregated nanoparticulate drug particles having a polymeric surface stabilizer and DOSS. Freeze dried powders can also be obtained by freeze drying aqueous dispersions of drug, polymeric surface stabilizer, and DOSS, which additionally contain a dissolved diluent such as lactose or mannitol. In these instances the freeze dried powders consist of particles of diluent, each of which contains at least one embedded drug nanoparticle combined with a polymeric surface stabilizer and DOSS.

Other known methods of processing liquid dispersions, and which can be employed in the present invention, include granulation, including but not limited to high shear granulation, fluid bed granulation, roto granulation, and melt granulation. Additional methods such as spray coating and extrusion spherization can also be used. Any other conventional method for drying or otherwise processing a liquid dispersion can also be used in the invention.

C. Methods of Using Nanoparticulate Drug Formulations Comprising One or More Polymeric Surface Stabilizers and DOSS

The solid dose nanoparticulate compositions of the present invention can be administered to humans and animals in any pharmaceutically acceptable manner, such as orally, rectally, pulmonary, intravaginally, locally (powders, ointments or drops), or as a buccal or nasal spray. Solid dosage forms for oral administration include capsules, tablets, pills, powders, pellets, and granules. In such solid dosage forms, the nanoparticulate drug is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures

thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Actual dosage levels of the drug in the nanoparticulate compositions of the invention may be varied to obtain an amount of active ingredient that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the drug, the desired duration of treatment, and other factors.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

EXAMPLE 1

The purpose of this example was to compare the redispersion properties of various solid dose nanoparticulate ketoprofen compositions in which DOSS is added to a nanoparticulate dispersion following milling and spray drying (rather than during the milling process). Ketoprofen. also known as m-benzoylhydratopic acid, is a nonsteroidal anti-inflammatory analgesic.

A ketoprofen nanoparticulate dispersion was prepared, having 15% ketoprofen, 1.5% PVP K29/32, and 0.075% SLS. The dispersion was prepared using a Dyno®—Mill (Type: KDL; Mfg.: Willy A Bachofen AG, Basel, Switzerland) equipped with a 150 cc batch chamber using a 500 μm milling media of type Polymill500® for 2 hrs at 10° C.

The ketoprofen nanoparticulate dispersion (ketoprofen NCD) was then spray dried with various excipients, as shown in Table 1, using a Büchi Mini Spray Dryer B-191 (B üchi Switzerland). Following spray drying, the redispersion properties of each spray dried ketoprofen powder were tested by measuring the ketoprofen particle size following redispersion and dilution with saturated ketoprofen solution, without sonication and following 1 minute sonication. Particle size was measured using a Horiba LA910 particle sizer. The results of the redispersion tests are also shown in Table 1, below.

TABLE 1

Redispersion Comparison of Nanoparticulate Ketoprofen Spray Dried Powder

	Composition Formula	Redispersed Particle Size (nm) (No sonication/1 min. sonication) Mean	D90*	% under 1000 nm
A	no additives	3801/3725	7697/7152	12.8/12.8
B	Drug:mannitol 1:1.2	6836/4050	15415/11173	41.8/52.2
C	Drug:Mannitol:DOSS 1:1.2:0.08	1860/1055	8785/453	84.6/90.1
D	Drug:Maltrin150** 1:1.2	20665/6104	38879/14479	9.2/26.6
E	Drug:Mannitol:DOSS 1:0.6:0.08	17149/2737	72756/10229	55.4/75.0
F	Drug:Xylitol 1:1	11241/5277	43502/12536	65.0/67.8
G	Drug:Xylitol:DOSS 1:1:0.08	1936/501	390/269	90.2/95.6
H	Drug:Mannitol:DOSS 1:1:0.08	4069/1944	15113/8313	72.6/80.0

TABLE 1-continued

Redispersion Comparison of Nanoparticulate Ketoprofen Spray Dried Powder

	Composition Formula	Redispersed Particle Size (nm) (No sonication/1 min. sonication) Mean	D90*	% under 1000 nm
I	Drug:Xylitol:DOSS 1:1:0.02	11469/2168	42333/7702	64.1/75.2
J	Drug:Mannitol:DOSS 1:1:0.08	2963/2004	10800/8011	72.2/77.5
K	Drug:Xylitol:DOSS 1:0.75:0.08	654/332	273/251	95.0/98.2

*90% of the particles are below this size.
**maltodextrin

The results dramatically show the effect DOSS has on the redispersibility of the spray dried nanoparticulate ketoprofen composition. Following redispersion, less than 13% of the ketoprofen particles of Composition A, lacking any additives (i.e., just spray dried ketoprofen NCD), had a particle size of less than a micron. Similarly, following redispersion less than 52.2% (following sonication) of the ketoprofen particles of Composition B, containing only mannitol as an additive, had a particle size of less than a micron. In contrast, following redispersion 90.1% (following sonication) of the ketoprofen particles of Composition C, containing mannitol and DOSS as additives, had a particle size of less than a micron. Thus, DOSS resulted in a 75% increase in the amount of particles having a particle size of under 1 micron following redispersion. This is significant as smaller drug particles result in greater bioavailability of the drug.

The amount of DOSS in relation to other excipients also affects the redispersion properties of the solid dose nanoparticulate drug composition. Thus, by varying the amount of DOSS and other excipients, redispersion of a solid dose nanoparticulate composition can be optimized. For example, Composition C, having a Drug:Mannitol:DOSS ratio of 1:1.2:0.08 showed 90.1% of the ketoprofen particles (following sonication) having a particle size of less than 1 micron following redispersion. However, Composition E, having a Drug:Mannitol:DOSS ratio of 1:0.6:0.08, showed 75.0% of the ketoprofen particles (following sonication) having a particle size of less than 1 micron following redispersion; Compositions H and J, having a Drug:Mannitol:DOSS ratios of 1:1:0.08, showed 80.0% and 77.5%, respectively, of the ketoprofen particles (following sonication) having a particle size of less than 1 micron following redispersion.

Similar results were obtained with spray dry excipients other than mannitol. For example, Composition F, having a Drug:Xylitol ratio of 1:1, showed 67.8% of the ketoprofen particles (following sonication) having a particle size of less than 1 micron following redispersion. In contrast, Compositions G and K, having Drug:Xylitol:DOSS ratios of 1:1:0.08 and 1:0.75:0.08, respectively, showed 95.6% and 98.2%, respectively, of the ketoprofen particles (following sonication) having a particle size of less than 1 micron following redispersion. This is an increase of 41% (Composition G) and 45% over the results obtained with Composition F, lacking DOSS.

This example demonstrates the effectiveness of adding DOSS to form a highly redispersible solid dose nanoparticulate composition, when DOSS is added following milling but before spray drying of the nanoparticulate dispersion. Other examples demonstrate the addition of DOSS to the nanoparticulate dispersion during milling. Thus, the time of addition of DOSS during preparation of the pharmaceu-

tical composition is not critical to the goal of obtaining a highly redispersible composition.

EXAMPLE 2

The purpose of this example was to evaluate the redispersion properties of a solid dose nanoparticulate ketoprofen composition comprising DOSS and a polymeric stabilizer in an electrolyte solution. This example differs from Example 1 in that DOSS is added directly to the nanoparticulate dispersion (NCD) during milling, followed by preparation of a solid dose composition.

A ketoprofen nanoparticulate dispersion was prepared, having the composition 5% ketoprofen, 1.% PVP K29/32, and 0.2% DOSS. The dispersion was prepared using a Dyno®—Mill (Type: KDL; Mfg.: Willy A Bachofen AG, Basel Switzerland) equipped with a 150 cc batch chamber using a 500 μm milling media of type Polymill500® for 2 hrs at 10° C.

The ketoprofen nanoparticulate dispersion (ketoprofen NCD) was then spray dried with mannitol, with a drug to mannitol ratio of 1:1 using a Büchi Mini Spray Dryer B-191 (Büchi Switzerland). The redispersion properties of the spray dried ketoprofen in water are shown below in Table 2.

TABLE 2

Redispersion Properties of Ketoprofen
Spray Dried NCD Containing DOSS in Water

Time (days)	Mean (no sonication)	Mean (1 min. sonication)	D_{50} (1 min. sonication)	D_{50} (no sonication)	D_{90} (1 min. sonication)	D_{90} (no sonication)
0	118	121	105	107	192	198
1	152	163	144	155	219	233

All measurements are in nanometers (nm).

The results of the redispersion test show excellent redispersion of the spray dried nanoparticulate ketoprofen composition comprising DOSS.

The redispersion properties of the same spray dried ketoprofen composition were then tested in electrolyte solutions, which mimic the conditions found in the human gastrointestinal tract. The results of these tests are shown in Table 3, below.

TABLE 3

Redispersion Properties of Ketoprofen
Spray Dried NCD Comprising DOSS in an Electrolyte Solution

Electrolyte Conc. (M)	Type	no sonic. Mean	No sonic. Small %	No sonic. Large %	1 min. sonic. Mean	1 min. sonic. Small %	No. sonic. Large %
0	—	172	100	0	182	100	0
0.001	HCl	535	97	3	166	100	0
0.01	HCl	176	100	0	188	100	0
0.1	HCl	17756	2	98	5908	8	92
0.001	NaCl	178	100	0	191	100	0
0.01	NaCl	151	100	0	163	100	0
0.1	NaCl	186	100	0	204	100	0

All particle sizes are in nanometers (nm).

"Small" particles are defined as those below 1 micron (1000 nm) and "large" particles are those above 1 micron. Electrolyte concentrations of 0.001 HCl, 0.01 HCl, and 0.1 HCl correspond to pH 3, pH 2, and pH 1, respectively. In the stomach, the pH ranges from slightly less than 2 (but typically greater than 1) up to 4 or 5. In the small intestine the pH can range from 4 to 6, and in the colon it can range from 6 to 8. Thus, a 0.01 N HCl concentration simulates typical acidic conditions found in the stomach. 0.1 M NaCl simulates the electrolyte concentration found throughout the body, including the intestine.

The results show that under acidic to neutral pH conditions, the nanoparticulate ketoprofen solid dose composition showed excellent redispersion properties, with 100% of the nanoparticulate particles having a redispersed particle size of less than 1 micron. In addition, under all but the most acidic conditions of 0.1 M HCl (which are not typically representative of human gastric pH), the nanoparticulate ketoprofen solid dose composition showed excellent redispersion properties, with almost 100% of the nanoparticulate particles having a redispersed particle size of less than 1 micron.

EXAMPLE 3

The purpose of this example was to evaluate the redispersion properties of a solid dose nanoparticulate MAP kinase inhibitor composition comprising DOSS and a polymeric stabilizer in electrolyte solutions.

5% (w/w) of Compound A, a MAP kinase inhibitor, 1% Plasdone® S630, and 0.2% DOSS were milled using a Dyno®—Mill (Type: KDL; Mfg.: Willy A Bachofen AG, Basel Switzerland) equipped with a 150 cc batch chamber using a 500 μm milling media of type Polymill500® for 3 hrs at 10° C.

The nanoparticulate MAP kinase inhibitor dispersion (NCD) was then spray dried at a drug to mannitol ratio of 1:1 using a Büchi Mini Spray Dryer B-191 (Büchi Switzerland). The redispersion properties of the spray dried MAP kinase inhibitor in electrolyte solutions are shown below in Table 4 and in FIG. 1. A Horiba LA910 particle sizer was used to

measure particle size. "Small" particles were defined as those below 1 micron and "large" particles were defined as those above 1 micron.

TABLE 4

Redispersion Properties of a MAP Kinase Inhibitor Spray Dried NCD Comprising DOSS and a Polymeric Stabilizer in an Electrolyte Solution

Electrolyte Conc. (M)	Type	no sonic. Mean	No sonic. Small %	No sonic. Large %	1 min. sonic. Mean	1 min. sonic. Small %	No. sonic. Large %
0	—	99	100	0	99	100	0
0.001	HCl	100	100	0	100	100	0
0.01	HCl	105	100	0	106	100	0
0.1	HCl	4708	23	77	1901	52	48
0.001	NaCl	103	100	0	103	100	0
0.01	NaCl	101	100	0	101	100	0
0.1	NaCl	105	100	0	105	100	0

All particle sizes are in nanometers (nm).

The results show that the solid dose nanoparticulate MAP kinase inhibitor composition, comprising DOSS and a polymeric stabilizer, showed excellent redispersion in all tested electrolyte media representative of in vivo conditions. Even at a higher acid concentration of 0.1 N HCl, the composition showed over 50% of the drug particles of the composition having a small particle size following 1 minute of sonication.

EXAMPLE 4

The purpose of this example was to evaluate the redispersion properties of a solid dose nanoparticulate angiogenesis inhibitor composition comprising DOSS and a polymeric stabilizer, which has been spray granulated with various excipients, in water and in electrolyte solutions.

Nanocrystalline dispersions (NCD) of an angiogenesis inhibitor, Compound C, were made by milling the ingredients shown for each composition in Table 7. Samples A and B were milled on a Netzch Mill (Netzsch Inc., Exton, Pa.), having a LMZ 2L chamber, for 11 hrs. 500 micron PolyMill media was used. Processing temperatures ranged from 11.6° C. to 27.4° C. Samples C–E were milled on a Dyno® Mill, having a 150 cc chamber, at a temperature of 10° C. for 3 hours, also using 500 micron PolyMill media.

Following milling, the additives listed in Table 5 were added to the nanoparticulate dispersion until dissolved, followed by spraying of the dispersion over a fluidized mannitol excipient, also provided in Table 5, to form a solid dose composition. A Glatt GPCG-1 fluid bed processor (Glatt Air Technologies, Inc., Ramsey, N.J.) was used for this process.

TABLE 5

Spray Granulated Nanoparticulate Angiogenesis Inhibitor Compositions

Sample	Formula	Particle Size of Nanocrystal Dispersion (nm)	Additives	Fluidized Mannitol
A	15% Drug + 3.75% PVP K29/32 and 0.15% SLS	mean 105 nm; D_{90} of 167 nm	Drug:mannitol ratio of 1:0.75	Pearlitol ® SD200
B	15% Drug + 3.75% PVP K29/32 and 0.15% SLS	mean 105 nm; D_{90} of 167 nm	Drug:mannitol ratio of 1:0.75	Pearlitol ® SD200
C	15% Drug + 3.75% PVP K29/32, 0.15% SLS, and 0.1% sodium ascorbate	mean of 101 nm; D_{90} of 165 nm	Drug:mannitol ratio of 1:0.75	Mannitol 35
D	15% Drug + 3.75% PVP K29/32, 0.15% SLS, and 0.1% sodium ascorbate	mean of 101 nm; D_{90} of 165 nm	Drug:mannitol ratio of 1:0.75	Mannitol 35
E	15% Drug + 3.75% PVP K29/32, 0.15% SLS, and 0.1% sodium ascorbate	mean of 101 nm; D_{90} of 165 nm	Drug:mannitol ratio of 1:0.75 and stabilizer DOSS ratio of 1:0.2	Mannitol 35

Each composition A–E, comprising drug/excipient granules, was then milled to a uniform particle size in a

Quadro Comill (Model 193; also called a cone mill, which comprises fixed stationary screens and a rotating impeller), to produce Compositions A2–E2. The milling process comprised passing the powder through the mill (one pass through, about 2–5 minutes).

The redispersibility, in water and various electrolyte solutions, was then measured for the solid dose nanoparticulate angiogenesis compositions, both Compositions A–E (unmilled) and A2–E2 (milled), as shown in Table 6.

TABLE 6

Redispersibility of Spray Granulated Nanoparticulate Angiogenesis Inhibitor Compositions (Milled and Unmilled Granulate Compositions)

		No Sonication			1 Min. Sonication		
Composition	Redisp. Media	Mean (nm)	D90 (nm)	% Under 1000 nm	Mean (nm)	D90 (nm)	% Under 1000 nm
A	water	5265	11776	26.2	1440	4717	70.8
(unmilled)	0.01 N HCl	12160	27244	9.4	3034	6997	36.1
	0.01 M NaCl	7487	15324	11.6	2274	6504	57.6
A2	water	5777	12463	23.0	2538	7547	62.9
(milled)	0.01 M N HCl	58519	236602	5.3	3573	7929	30
	0.01 M NaCl	8341	17698	11	1975	5366	54.9
B	water	8222	18365	18.5	4368	9033	51.5
(unmilled)	0.01 N HCl	83643	264545	4.8	4238	9458	26.3
	0.01 M NaCl	14863	331391	8	2579	6561	45.8
B2	water	18897	55523	14.2	2691	7294	50
(milled)	0.01 N HCl	44037	103747	4.1	5161	1771	22.4
	0.01 M NaCl	13514	29820	6.8	2547	6163	42.1
C	water	3124	8088	46.9	422	645	93.4
(unmilled)	0.01 N HCl	6713	14117	16.6	2471	6285	47
	0.01 M NaCl	4103	9426	30.6	904	3006	80.4
C2	water	3150	8427	49	1071	3602	83.6
(milled)	0.01 N HCl	8728	19180	17.1	3039	7626	43.3
	0.01 M NaCl	4544	9896	25.5	1278	4345	75
D	water	3094	7865	44.8	342	569	97.3
(unmilled)	0.01 N HCl	9630	21697	14.8	2762	7043	45.3
	0.01 M NaCXl	4295	8561	20.6	1475	5034	73.6
D2	water	2162	5885	54.4	295	488	98.7
(milled)	0.01 N HCl	8885	20181	16.9	1982	5087	51.7
	0.01 M NaCl	4410	8710	19	1066	3420	75.9
E	water	2186	7520	69.9	384	614	98.3
(unmilled)	0.01 N HCl	2161	7812	73.4	297	492	99
	0.01 M NaCl	2544	8755	68.1	357	588	98.5
E	water	2711	9141	66.6	436	672	93.6
(milled)	0.01 N HCl	2014	7608	75.9	291	483	99.1
	0.01 M NaCl	2203	8075	74.1	292	484	99

Only Sample E comprises DOSS. The redispersibility results showed that only this sample showed substantially improved redispersion in electrolyte media, with a redispersibility of 99.1% in 0.01 N HCl and 99% in 0.01 M NaCl. In contrast, Samples A–D showed redispersibility in 0.01 N HCl of from 22.4% (Sample B2) to 51.7% (Sample D2), and a redispersibility in 0.01 N HCl of from to 42.1% (Sample B2) to 80.4% (Sample C). The results are dramatic as the only difference between Sample E and Samples C and D was the presence (Sample E) or absence (Samples C and D) of DOSS.

The results demonstrate the dramatically superior redispersibility properties of a solid dose nanoparticulate formulation comprising DOSS.

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

We claim:

1. A solid dose nanoparticulate composition comprising:

(a) an active agent which has a solubility in a liquid dispersion medium of less than about 10 mg/mL;

(b) at least one polymeric surface stabilizer adsorbed on the surface of the active agent; and

(c) about 0.1% to about 20% w/w, of dioctyl sodium sulfosuccinate (DOSS),

wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 1 micron, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 5 microns.

2. The composition of claim 1, wherein the active agent is present in an amount of about 99.8% to about 0.1% (w/w).

3. The composition of claim 1, wherein the active agent is present in an amount of about 80% to about 5% (w/w).

4. The composition of claim 1, wherein the active agent is present in an amount of about 50% to about 10% (w/w).

5. The composition of claim 1, wherein the at least one polymeric surface stabilizer is present in an amount of about 0.01% to about 90% (w/w).

6. The composition of claim 1, wherein the at least one polymeric surface stabilizer is present in an amount of about 1% to about 75% (w/w).

7. The composition of claim 1, wherein the at least one polymeric surface stabilizer is present in an amount of about 10% to about 60% (w/w).

8. The composition of claim 1, wherein DOSS is present in an amount of about 1% to about 10% (w/w).

9. The composition of claim 1, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 800 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 4 microns.

10. The composition of claim 1, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 600 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 3 microns.

11. The composition of claim 1, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 400 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 2 microns.

12. The composition of claim 1, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 200 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 1 micron.

13. The composition of claim 1, wherein the active agent is selected from the group consisting of a crystalline phase drug, a semi-crystalline phase drug, and an amorphous phase drug.

14. The composition of claim 1, wherein the active agent is selected from the group consisting of proteins, peptides, nucleotides, anti-obesity drugs, nutriceuticals, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

15. The composition of claim 1, wherein the at least one polymeric surface stabilizer is selected from the group consisting of polyvinylpyrrolidone (PVP), cellulose ethers, polysaccharides, random copolymers of vinyl acetate and vinyl pyrrolidone, polyvinyl alcohol, and copolymers of vinyl acetate and vinyl alcohol.

16. The composition of claim 15, wherein the at least one polymetric surface stabilizer is selected from the group consisting of hydroxypropyl cellulose, hydroxypropyl methylcellulose, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, dextrin, guar gum, starch, and a copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate in a mass proportion of 3:2.

17. A method of making a solid dose nanoparticulate composition having a high redispersibility upon administration to a mammal comprising:

(a) dispersing particles of an active agent which has a solubility in a liquid dispersion medium of less than about 10 mg/mL in a liquid dispersion medium;

(b) applying mechanical means in the presence of grinding media to reduce the effective average particle size of the active agent in the liquid dispersion medium to less than about 1 micron, wherein at least one polymeric surface stabilizer and dioctyl sodium sulfosuccinate are added to the liquid dispersion medium before or after milling;

(c) drying the nanoparticulate dispersion comprising an active agent, at least one polymeric surface stabilizer, and dioctyl sodium sulfosuccinate; and

(d) formulating the dry nanoparticulate composition into a solid dose form for administration,

wherein upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 5 microns.

18. The method of claim 17, wherein the active agent is present in an amount of about 99.8 to about 0.1% (w/w).

19. The method of claim 17, wherein the at least one polymeric surface stabilizer is present in an amount of about 0.01% to about 90% (w/w).

20. The method of claim 17, wherein DOSS is present in an amount of about 0.1% to about 20% (w/w).

21. The method of claim 17, wherein DOSS is present in an amount of about 1.0% to about 10% (w/w).

22. The method of claim 17, wherein the active agent is selected from the group consisting of a crystalline phase drug, a semi-crystalline phase drug, and an amorphous phase drug.

23. The method of claim 17, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 800 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 4 microns.

24. The method of claim 17, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 600 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 3 microns.

25. The method of claim 17, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said

poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 400 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 2 microns.

26. The method of claim **17**, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 200 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 1 micron.

27. A method of treating a patient in need with a solid dose nanoparticulate composition having high redispersibility of the nanoparticulate composition upon administration to a mammal, comprising administering to a patient in need a therapeutically effective amount of a solid dose nanoparticulate composition comprising:

(a) an active agent which has a solubility in a liquid dispersion medium of less than about 10 mg/mL;

(b) at least one polymeric surface stabilizer adsorbed on the surface of the active agent; and

(c) about 0.1% to about 20% w/w of dioctyl sodium sulfosuccinate (DOSS),

wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 1 micron, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 5 microns.

28. The method of claim **27**, wherein the active agent is present in an amount of about 99.8 to about 0.1% (w/w).

29. The method of claim **27**, wherein the at least one polymeric surface stabilizer is present in an amount of about 0.01% to about 90% (w/w).

30. The method of claim **27**, wherein DOSS is present in an amount of about 1.0% to about 10% (w/w).

31. The method of claim **27**, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 800 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 4 microns.

32. The method of claim **27**, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 600 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 3 microns.

33. The method of claim **27**, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 400 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 2 microns.

34. The method of claim **27**, wherein the effective average particle size of the nanoparticulate dispersion prior to incorporation in the solid dose formulation, comprising said poorly soluble active agent and at least one polymeric surface stabilizer, is less than about 200 nm, and upon reconstitution in media representative of human physiological conditions, the solid dose nanoparticulate composition redisperses such that 90% of the active agent particles have a particle size of less than about 1 micron.

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3. U.S. Patent No. 5,552,160 to Liversidge et al.

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Liversidge et al.

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[45] **Date of Patent: *Sep. 3, 1996**

[54] **SURFACE MODIFIED NSAID NANOPARTICLES**

[75] Inventors: **Gary G. Liversidge**; **Philip Conzentino, Jr.**, both of West Chester; **Kenneth C. Cundy**, Pottstown; **Pramod P. Sarpotdar**, Malvern, all of Pa.

[73] Assignee: **NanoSystems L.L.C.**, Collegeville, Pa.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,145,684.

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[22] Filed: **Mar. 13, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 897,193, Jun. 10, 1992, abandoned, which is a continuation-in-part of Ser. No. 647,105, Jan. 25, 1991, Pat. No. 5,145,684.

[51] **Int. Cl.**[6] **A61K 9/14**

[52] **U.S. Cl.** **424/489**; 424/450; 424/495; 424/499

[58] **Field of Search** 424/489, 450, 424/499, 495

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Primary Examiner—Thurman K. Page
Assistant Examiner—William E. Benston, Jr.
Attorney, Agent, or Firm—Rudman & Balogh

[57] **ABSTRACT**

Dispersible particles consisting essentially of a crystalline NSAID having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 400 nm. Pharmaceutical compositions containing the particles exhibit reduced gastric irritation following oral administration and/or hastened onset of action.

13 Claims, No Drawings

SURFACE MODIFIED NSAID NANOPARTICLES

This application is a continuation of U.S. patent application Ser. No. 07/897,193, filed Jun. 10, 1992, now abandoned, which was a continuation-in-part of U.S. patent application Ser. No. 647,105, filed Jan. 25, 1991, now U.S. Pat. No. 5,145,684, issued Sep. 8, 1992.

BACKGROUND OF INVENTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used and therapeutically effective groups of drugs. However, gastric irritation problems constitute the most frequently recognized adverse side effect following oral administration of NSAIDs. Such side effects are well recognized and must be weighed against the clinical efficacy of the drugs.

A great amount of research has been undertaken in an attempt to understand the underlying mechanism responsible for these effects. For example, Cioli et al, *Tox. and Appl. Pharm.*, 50, 283–289 (1979) suggest that gastrointestinal lesions in laboratory animals resulting from the oral administration of acidic NSAIDs may depend on two different mechanisms: a local action exerted by contact with the gastric mucosa and a generalized/centrally mediated (systemic) action, taking place following oral administration.

More recently, Price et al, *Drugs* 40 (Suppl. 5):1–11, 1990, suggest that NSAID-induced gastric damage occurs as a result of NSAID-mediated direct and indirect acidic damage followed almost simultaneously by the deleterious systemic effect of prostaglandin inhibition.

A variety of strategies have been used in the management of NSAID-induced gastric damage. These include: 1) the development and use of NSAIDs with less toxic potential; 2) the reduction or elimination of the agent that actually causes the injury; and 3) the enhancement of the mucosal defense. However, these approaches have not proven entirely successful.

For example, the most effective means of preventing gastric damage, i.e., by eliminating the primary aetiological agent is rarely feasible with NSAIDs inasmuch as patients with severe inflammatory disease are rarely able to cease using these drugs. Although selection of less toxic NSAIDs should prove useful, the only practical solution, at present, is to treat the NSAID induced gastric damage. Misoprostol (a methylated prostaglandin E_1) has been approved by the FDA for use in preventing NSAID gastropathy. However, Misoprostol is expensive, must be administered multiple times daily and can cause unacceptable side effects.

Thus it would be highly desirable to provide NSAID formulations that can exhibit a reduction in gastric irritation. Moreover, it would be desirable to provide NSAID formulations exhibiting hastened onset of action.

SUMMARY OF THE INVENTION

We have discovered that pharmaceutical compositions containing surface modified NSAID nanoparticles exhibit reduced gastric irritation following oral administration and/or more rapid onset of action.

More particularly, in accordance with this invention, there are provided particles consisting essentially of an NSAID having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an average particle size of less than about 400 nm.

This invention further provides a pharmaceutical composition comprising the above-described particles and a pharmaceutically acceptable carrier.

In another embodiment of the invention, there is provided a method of treating a mammal comprising administering to the mammal the above-described pharmaceutical composition.

In yet another embodiment of the invention, there is provided a method of preparing the above-described particles comprising the steps of dispersing an NSAID in a liquid dispersion medium and wet grinding the NSAID in the presence of rigid grinding media, wherein the pH of said medium is maintained within the range of from 2 to 6.

In further embodiments of the invention, there are provided methods of reducing gastric irritation and/or hastening the onset of action which include administering the above-described pharmaceutical composition to a mammal.

It is an advantageous feature of this invention that pharmaceutical compositions containing NSAIDs are provided which exhibit reduced gastric irritation following oral administration.

It is another advantageous feature of this invention that pharmaceutical compositions are provided exhibiting hastened onset of action.

Other advantageous features will become readily apparent upon reference to the following description of preferred embodiments.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention is based partly on the discovery that surface modified nanoparticles comprising an NSAID, e.g., naproxen, demonstrate reduced gastric irritation and/or a more rapid onset of action following oral administration. While the invention is described herein primarily in connection with its preferred class of drugs, i.e., NSAIDs, it is also useful in conjunction with other classes of drug substances, e.g., antibiotics, quinolones, antilipemics and roentgenographics.

The particles of this invention comprise an NSAID. The NSAID exists as a discrete, crystalline phase. The crystalline phase differs from an amorphous or non-crystalline phase which results from conventional solvent precipitation techniques, such as described in U.S. Pat. No. 4,826,689. The NSAID can be present in one or more suitable crystalline phases.

The invention can be practiced with a wide variety of NSAIDs. However, the NSAID must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the NSAID has a solubility in the liquid dispersion medium, e.g., water, of less than about 10 mg/ml, and preferably of less than about 1 mg/ml at processing temperature, e.g., room temperature. The preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which the NSAID is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

The NSAIDs useful in the practice of this invention can be selected from suitable acidic and nonacidic compounds.

Suitable acidic compounds include carboxylic acids and enolic acids. Suitable nonacidic compounds include, for example, nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine and dapsone.

Suitable carboxylic acid NSAIDs include, for example, salicylic acids and esters thereof, such as aspirin, phenylacetic acids such as diclofenac, alclofenac and fenclofenac, and carbo- and heterocyclic acetic acids such as etodolac, indomethacin, sulindac, tolmetin, fentiazac and tilomisole; propionic acids, such as carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen; and fenamic acids, such as flufenamic, mefenamic, meclofenamic and niflumic.

Suitable enolic acid NSAIDs include, for example, pyrazolones such as oxyphenbutazone, phenylbutazone, apazone and feprazone, and oxicams such as piroxicam, sudoxicam, isoxicam and tenoxicam.

The above-described NSAIDs are known compounds and can be prepared by techniques known in the art.

In particularly preferred embodiments of the invention, the NSAID is naproxen, indomethacin or ibuprofen.

The particles of this invention contain an NSAID as described above having a surface modifier adsorbed on the surface thereof. Useful surface modifiers are believed to include those which physically adhere to the surface of the NSAID but do not chemically bond to the NSAID.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, polaxomers, such as Pluronic™ F68 and F108, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine available from BASF, dextran, lecithin, Aerosol OT™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol™ P, which is a sodium lauryl sulfate, available from DuPont, Triton™ X200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 20 and Tween 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, Crodesta™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodesta SL-40, which is available from Croda, Inc., and SA90HCO, which is $C_{18}H_{37}$-$CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include polyvinylpyrrolidone, Pluronic F-68, and lecithin.

The surface modifier is adsorbed on the surface of the NSAID in an amount sufficient to maintain an effective average particle size of less than about 400 nm. The surface modifier does not chemically react with the NSAID or itself. Furthermore, the individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages.

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles have a number average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments of the invention, the effective average particle size is less than about 300 nm. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size of less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm.

The particles of this invention can be prepared in a method comprising the steps of dispersing an NSAID in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the NSAID to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

A general procedure for preparing the particles of this invention is set forth below. The NSAID selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse NSAID selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of the NSAID is greater than about 100 μm, then it is preferred that the particles of the NSAID be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse NSAID selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the NSAID in the liquid medium can vary from about 0.1–60%, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be-present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the

dispersion to less than 400 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the NSAID and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the NSAID conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, a planetary mill, media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. However, zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. Further, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 2.5 g/cm^3.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the NSAID. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

Milling must be carried out under acidic conditions, at a pH of from 2–6, preferably 3–5. The preferred pH depends, e.g., on the acidity and solubility of the particular NSAID selected. Acid resistant milling equipment is highly preferred, e.g., equipment fabricated of high grade stainless steel, e.g., grade 316 SS, or equipment coated with an acid resistant coating.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

The relative amount of the NSAID and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular NSAID and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the surface area of the NSAID, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square meter surface area of the NSAID. The surface modifier can be present in an amount of 0.1–90%, preferably 0.5–80%, and more preferably 1–60% by weight based on the total weight of the dry particle.

A simple screening process has been developed whereby compatible surface modifiers and NSAIDs can be selected which provide stable dispersions of the desired particles. First, coarse particles of an NSAID are dispersed in a liquid in which the NSAID is essentially insoluble, e.g., water at 5% (w/v) and milled for 120 hours in a roller mill under the following milling conditions:

Grinding vessel: 8 oz. (250 ml) glass jar

Available volume of grinding vessel: 250 ml

Media volume: 120 ml

Media type: 1.0 mm pre-cleaned zirconium oxide beads (distributed by Zircoa, Inc.)

Milling time: 120 hours

Slurry volume: 60 ml

RPM: 92

Room Temperature pH: 4.0 (adjusted with HCl or NaOH, if necessary)

The slurry is separated from the milling media by conventional means, e.g., by pouring the slurry out of the vessel, or by using a pipette. The separated slurry is then divided into aliquots and surface modifiers are added at a concentration of between 2 and 50% by weight based on the total combined weight of the NSAID and surface modifier. The dispersions are then sonicated (1 minute, 20 kHz) or vortexed using a multitubed vortexer for one minute, to disperse agglomerates and subjected to particle size analysis, e.g., by photon correlation spectroscopy and/or by examination under an optical microscope (1000× magnification). If a stable dispersion is observed, then the process for preparing the particular NSAID surface modifier combination can be optimized in accordance with the teachings above. By stable it is meant that the dispersion exhibits no flocculation or particle agglomeration visible to the naked eye and, preferably, when viewed under the optical microscope at 1000×, at least 15 minutes, and preferably, at least two days or longer after preparation. In addition, preferred particles exhibit no flocculation or agglomeration when dispersed in 0.1N HCl or simulated GI fluid (USP).

The resulting dispersion is stable and consists of the liquid dispersion medium and the above-described particles. The dispersion of surface modified NSAID nanoparticles can be spray coated onto sugar spheres or onto a pharmaceutical excipient in a fluid-bed spray coater by techniques well known in the art.

Pharmaceutical compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art. These include non-toxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral admin-

istration in solid or liquid form, for rectal administration, and the like. A method of treating a mammal in accordance with this invention comprises the step of administering to the mammal in need of treatment an effective amount of the above-described pharmaceutical composition. The selected dosage level of the NSAID for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore, depends upon the particular NSAID, the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

It is a particularly advantageous feature that the pharmaceutical compositions of this invention exhibit reduced gastric irritation and/or more rapid onset of action as illustrated in the examples that follow.

The following examples further illustrate the invention.

Example 1

A nanoparticulate naproxen dispersion (Formula 1) was prepared in a roller mill as follows. A 250 ml glass jar was charged with 120 ml of 1.0 mm pre-cleaned Zirconium oxide beads (Zirbeads XR, available from Zircoa Inc., having a nominal diameter of 1.0 mm), 60 g of an aqueous slurry containing 3 g naproxen (5% by weight), purchased from Sigma, St. Louis, Mo., particle size 20–30 μm, and 1.8 g (3% by weight) Pluronic F-68, purchased from BASF Fine Chemicals, Inc., as the surface modifier. The beads were pre-cleaned by rinsing in 1N H_2SO_4 overnight followed by several rinses with deionized water. The batch was rolled at 92 RPM for a total of 120 hours. The dispersion was stable when a portion was added to 0.1N HCl. The average particle size measured by photon correlation spectroscopy was 240–300 nm.

A control formulation of naproxen was prepared by adding 5% (w/v) unmilled naproxen to 3% Pluronic F-68. The suspension was vortexed and sized. The particle size range was 20–30 μm.

The concentration of naproxen in both formulations was 50 mg/mL (w/v). Both formulations were diluted with 3% Pluronic F-68 to a dosing concentration of 10 mg/mL for oral administration.

Male Sprague-Dawley rats were maintained in accordance with the conditions set forth in "Guide for the Care and Use of Laboratory Animals", NIH Publication 86-23 The temperature was maintained at 22±1° C. and the relative humidity was 50±10%, with a 12 hour light/dark cycle. Rats were provided laboratory chow and water. The rats (250–350 g) were anesthetized with a 55 mg/kg intraperitonal injection of Nembutal (sodium pentobarbital). The external Jugular veins were chronically cannulated to facilitate removal of blood samples. Prior to administration of naproxen, the rats were allowed to recover for 24 hours with water ad libitum.

The rats were anesthetized, with Metofane, orally gavaged with the above-described formulations and placed in a restraint device. Blood samples (100 μl) were obtained via the jugular vein at 0 (pre-administration), 5, 10, 15, 30, 45, 60, 75, 90, 120, 180 and 240 minutes following administration of naproxen and collected in heparinized tubes. Plasma (50 μl) was obtained immediately and placed on ice. Plasma samples (50 μl) were mixed with 130 μl of acetonitrile and 20 μl of a standard solution (20 μg/ml indomethacin) and vortexed to precipitate protein. Samples were centrifuged and the supernatants removed, placed in vials, and analyzed by HPLC. The Separation of naproxen was carried out on an analytical column (Waters Novapak C18; 15 cm×4 mm, 5μ).

At the end of the experiment (240 min.) the rats were euthanized by an I.V. bolus injection of Nembutal via the jugular vein. The stomachs were removed and cut along the line of greater curvature from the duodenum to the pyloric sphincter. The stomachs were then spread flat and pinned out on dissecting dishes, and washed with 0.9% NaCl.

The evaluation and counting of stomach irritations (erosion/lesion/ulcer) were conducted by a modification of arbitrary scoring systems (Cioli et al, *Tox. and Appl. Pharm.*, 1979, 50:283–289 and Beck et al, *Arch. Toxicol.*, 1990; 64: 210–217) correcting for various degrees of severity as noted below. Differences in severity index have been associated with the gastropathology present on the stomach following oral administration of NSAIDs (Balaa, *Am. Journ. Med. Sci.*, 1991, 301:272–276 and Lanza et al; *Dig. Dis. and Sci.*, 1990; 35:12).

Each stomach irritation was measured in length (or diameter) using a 10 mm surgical ruler. The length of the irritations ranged from 0.25 mm to 10.0 mm. Irritations less than 0.25 mm were classified as pinpoint. The irritations were categorized by color as an evaluation of severity. Irritations red in appearance were rated as mild and assigned a severity value of 1. Brown irritations were rated as moderately severe and assigned a value of 2. Irritations which appeared black were rated as the most severe and given a severity value of 3. A score for each irritation was determined by multiplying the length value and the point severity level. The sum total for all irritations on a given stomach was identified as the total irritation score.

Table 1 shows the mean values for the stomach irritations induced by naproxen in the Control formulation and Formulation 1 of this invention. As indicated by the data, the formulation of this invention exhibited a reduction in stomach irritation scores compared to the control (p=0.099). It was concluded that the formulation of this invention exhibits reduced gastric irritation following oral administration as compared to the control.

TABLE 1

Rat No.	Control (n = 6)	Formulation 1 (n = 8)
1	293	43
2	200	139
3	133	149
4	140	80
5	110	129
6	101	163
7		54
8		98
Mean	163	107
SEM	30	16

Surprisingly, the formulation of this invention when administered orally induced a similar level of gastric irritation compared to the same formulation administered parenterally, i.e., I.V. Thus, the formulation of this invention appears virtually devoid of a direct irritant effect on the stomach of a rat.

A statistical comparison of the pharmacokinetic plasma parameters Cmax (peak plasma concentration), Tmax (time to peak plasma concentration) and relative bioavailability ($AUC_{(0\text{-}240\ min)}$—from Area Under the Curve values from 0–240 minutes) for Formulation 1 of this invention and the control calculated by the trapezoidal method is set forth below.

	Mean ± SEM	
	Control	Formulation 1
Cmax (μg/ml)	126 ± 4 (n = 5)	187 ± 19 (n = 6)
Tmax (min)	34 ± 3 (n = 5)	24 ± 5 (n = 6)
$AUC_{(0-240\ min)}$ (μg × min/ml)	15,228 ± 994 (n = 5)	19,062 ± 573 (n = 3)

The data indicate that the time to peak plasma concentration were lower for the formulation of this invention compared to the control (p=0.15) and both the relative bioavailability and peak plasma concentrations were significantly higher for the formulation of this invention compared to the control (p=0.03) and (p=0.02), respectively. The increase in apparent rate of absorption clearly suggests enhanced onset of action.

Example 2

The preparation of Example 1 was repeated except that 5% by weight polyvinylpyrrolidone was used in place of the Pluronic F-68. The average particle size was 250 mm.

Examples 3–8 illustrate the preparation of nanoparticulate ibuprofen.

Example 3

Nanoparticulate ibuprofen was prepared in a planetary mill (Pulverisette-7, manufactured by Fritsch, Gmbh) containing two 25 ml bowls. The initial charge (per bowl) included 12.5 ml of 1 mm pre-cleaned zirconium oxide beads and 6.25 ml of an aqueous slurry containing 100 mM HCl, 3% (w/v) ibuprofen, and 2% (w/v) Pluronic F-68 as the surface modifier. The ibuprofen formulation was milled for 24 hours at 325 RPM. The resulting dispersion was stable when a portion was added to simulated gastric fluid, i.e., 2 g NaCl, 3.2 g pepsin, 7 ml HCl, and H_2O to 1 liter, pH=1.2. The average particle size measured by photon correlation spectroscopy was 253 nm.

Example 4

Example 3 was repeated except that the initial charge included 1% Tween 20 and the milling time was 17 hours. The average particle size was 263 nm.

Example 5

Example 3 was repeated except that the milling time was 4 hours. The average particle size was 314 nm.

Example 6

Example 3 was repeated except that the surface modifier in the initial charge was 1% (w/v) of a 1:2 by weight mixture of Tween 20 and Span 20, and the milling time was 20 hours at 175 RPM. The average particle size was 294 nm.

Example 7

Example 3 was repeated except that the initial charge included 0.25% (w/v) tyloxapol as the surface modifier and 10 mM HCl . The charge was milled for 20 hours at 175 RPM in a refrigerated (5° C.) area. The average particle size was 344 nm.

Example 8

Example 7 was repeated except that Tween 20 was used in place of the tyloxapol. The average particle size was 351 nm.

Examples 9–12 illustrate the preparation of nanoparticulate indomethacin.

Example 9

Nanoparticulate indomethacin was prepared in a roller mill as follows. A 250 ml bottle was charged with 125 ml of 1.0 mm pre-cleaned ZrO_2 beads, 200 gm of an aqueous slurry containing 10 gms indomethacin (5% by weight) and 2 gms Vinol 205, a polyvinylalcohol (1% by weight). A batch size of 200 gms was used to reduce air space in the bottle to minimize the formation of foam. The batch was rolled at 88.5 RPM for a total of 240 hours. The dispersion was stable in 0.1N HCl and simulated gastric fluid as described in Example 3 above. The average particle size measured by photon correlation spectroscopy was 331 nm.

Example 10

Example 9 was repeated except that polyvinylpyrrolidone was used in place of the polyvinylalcohol. The average particle size was 216 nm.

Example 11

Example 9 was repeated except that Pluronic F-68 was used in place of the polyvinylalcohol. The average particle size was 228 nm.

Example 12

Example 9 was repeated except that Pluronic F-108 was used in place of the polyvinylalcohol. The average particle size was 235 nm.

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed is:

1. Particles consisting essentially of 99.9–10% by weight of crystalline NSAID having a solubility in water of less than 10 mg/ml, said NSAID having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an average particle size of less than about 400 nm.

2. The particles of claim 1 having an effective average particle size of less than 300 nm.

3. The particles of claim 1 wherein said surface modifier is present in an amount of 0.5 to 80% by weight based on the total weight of the dry particle.

4. The particles of claim 1 wherein said NSAID is selected from nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, dapsone, aspirin, diflunisal, benorylate, fosfosal, diclofenac, alclofenac, fenclofenac, etodolac, indomethacin, sulindac, tolmetin, fentiazac, tilomisole, carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen, flufenamic, mefenamic, meclofenamic, niflumic, oxyphenbutazone, phenylbutazone, apazone and feprazone, piroxicam, sudoxicam, isoxicam and tenoxicam.

5. The particles of claim 1 wherein said NSAID is selected from naproxen, indomethacin and ibuprofen.

6. The particles of claim 1 wherein said surface modifier

is selected from polyvinylpyrrolidone and a block copolymer of ethylene oxide and propylene oxide.

7. Particles according to claim 1 consisting of naproxen having a block copolymer of ethylene oxide and propylene oxide adsorbed on the surface thereof in an amount sufficient to maintain an average particle size of less than about 400 nm.

8. Particles according to claim 1 consisting essentially of naproxen having polyvinylpyrrolidone adsorbed on the surface thereof in an amount sufficient to maintain an average particle size of less than about 400 nm.

9. A pharmaceutical composition comprising the particles of claim 1 and a pharmaceutically acceptable carrier.

10. A method of treating a mammal comprising administering to the mammal an effective amount of the pharmaceutical composition of claim 9.

11. A method of reducing gastric irritation following oral administration to a mammal of a pharmaceutical composition comprising an NSAID, said method comprising administering the pharmaceutical composition of claim 9.

12. A method of hastening onset of action following administration to a mammal of a pharmaceutical composition an NSAID, said method comprising administering the pharmaceutical composition of claim 9.

13. A method of hastening onset of action following administration to a mammal of pharmaceutical composition, said method comprising administering said pharmaceutical composition in the form of particles consisting essentially of 99.9–10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an average particle size of less than about 400 nm.

* * * * *

4. Singh et al., *Analytical Profiles of Drug Substances and Excipients*, 28: 197-249 (2001)

NIMESULIDE

Amarjit Singh[1], Paramjeet Singh[1], Vijay K. Kapoor[2]

(1) Research & Development Centre
Panacea Biotec Ltd.
P.O. Lalru 140 501
India

(2) University Institute of Pharmaceutical Sciences
Panjab University
Chandigarh 160 014
India

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Contents

1. Description

1.1 Nomenclature

1.1.1 Systematic Chemical Name

4′-Nitro-2′-phenoxymethanesulphonanilide [1]

N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide [2]

4-nitro-2-phenoxymethanesulfonanilide [2]

(Methylsulfonyl)(4-nitro-2-phenoxyphenyl)amine [3]

1.1.2 Nonproprietary Names [1, 2]

Nimesulide

1.1.3 Proprietary Names [1, 2, 4]

Algolider; Antifloxil; Aulin; Eudolene; Fansidol; Flogovital; Flolid; Guaxan; Laidor; Ledoren, Ledoven; Mesid; Mesulid; MF 110; Nide; Nidol; Nimedex; Nimesulene; Nims; Nimulid; Nisal; Nisulid; Remov; Resulin; Sulide; Teonim

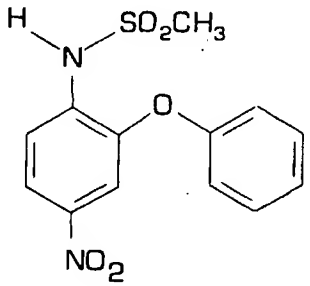
1.2 Formulae

1.2.1 Empirical Formula, Molecular Weight, CAS Number

$C_{13}H_{12}N_2O_5S$ [MW = 308.31]

CAS number = 51803-78-2

1.2.2 Structural Formula



1.3 Elemental Analysis

The calculated elemental composition is as follows:

carbon:	50.64%
hydrogen:	3.92%
oxygen:	25.95%
nitrogen:	3.92%
sulfur:	10.40%

1.4 Appearance

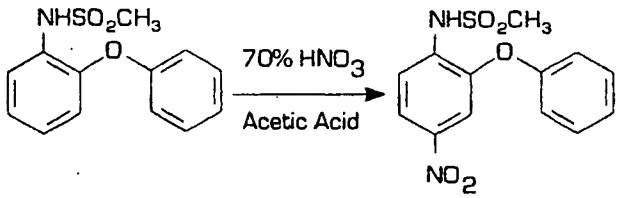
Nimesulide is a light yellow crystalline powder, which is practically odorless.

1.5 Uses and Applications

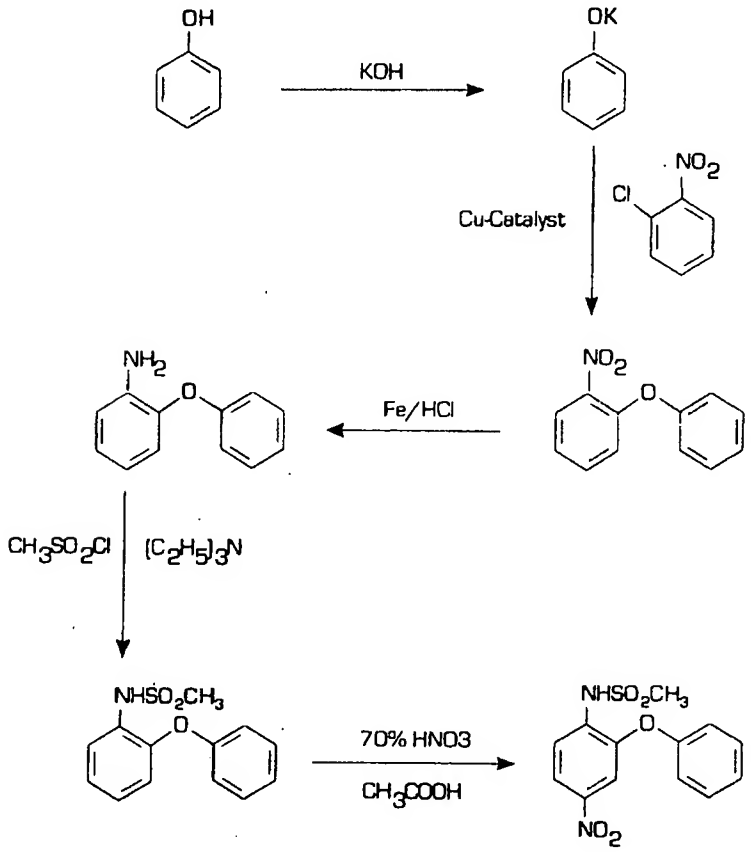
Nimesulide has analgesic, anti-inflammatory, and antipyretic properties, acting as an inhibitor of prostaglandin synthetase and platelet aggregation. It is given in doses of up to 200 mg twice daily by mouth for inflammatory conditions, fever, and pain.

2. Methods of Preparation

Nimesulide has been synthesized by Moore and Harrington [5] in 1974. The procedure involved the dissolution of 2-phenoxy-methane sulfonanilide in glacial acetic acid (by warming), followed by the dropwise addition of an equimolar amount of 70% nitric acid with continuous stirring. After heating the mixture on a steam bath for four hours, the final product is obtained by pouring the mixture in water. At this point, Nimesulide is precipitated.



An alternate route of synthesis (Scheme 2) has been developed [6] which starts with *o*-chloronitrobenzene and phenol. Nimesulide is reported [7] to be prepared in four steps in 61% overall yield from *o*-chloronitrobenzene and phenol.



A series of compounds structurally related to Nimesulide have been prepared in which *p*-nitrophenyl moiety has been replaced by pyridine and pyridine *N*-oxide [8]. In another modification, the p-nitro group of Nimesulide was substituted by a cyano and 1*H*-tetrazol-5-yl groups. Analogs were also prepared where the methanesulfonamido group was replaced by an acetamido group. However all such modifications were found to be detrimental to the activity.

Nimesulide analogs have been prepared that exhibit inhibition of bladder cancer in animal models [9], and as prophylactics and therapeutics for ischemia-reperfusion injury [10]. A number of Nimesulide analogs that act as anti-inflammatory agents have also been reported [11-17].

3. Physical Properties

3.1 Ionization Constants

Nimesulide is characterized by a single ionization constant associated with dissociation of the -NH proton of the sulfonanilide group. Various pKa values have been reported in the literature: 5.9 [38], 6.46 [39], 6.50 [40], and 6.56 [41]. These values clearly indicate the acidic nature of the drug.

A method for automated pKa determination by capillary zone electrophoresis has been described [42].

3.2 Solubility Characteristics

Nimesulide is soluble in moderately polar solvents such as dichloromethane and acetone. The solubility is diminished in solvents of high polarity such as methanol. The solubility of Nimesulide in water is reported to be 0.01 mg/mL [22], which becomes enhanced by an increase in the pH of the aqueous solution. This is essentially due to deprotonation and ionization of sulfonanilide group. Table 1 provides the solubility characteristics of Nimesulide in buffers over the pH range of 4 to 11, as well as in a variety of solvents.

To overcome the problem of very poor water solubility, various inclusion complexes with β-cyclodextrin [23-28] and hydroxypropyl β-cyclodextrin [28] have been prepared. A Nimesulide-β-cyclodextrin complex has been reported to have an aqueous solubility of about 16 mg/mL [22]. To assess the efficacy and tolerability of single doses of Nimesulide-β-cyclodextrin, a comparative study of the complex and Nimesulide has been carried out in patients with dental pain following surgical procedures [29]. *In-vitro* and *in-vivo* studies on the sodium Nimesulide-β-cyclodextrin inclusion complex have been carried out [30].

Table 1

Solubility Characteristics of Nimesulide

Solvent System	Solubility (mg/mL)
Buffer pH 4.0	0.068
Buffer pH 5.0	0.0078
Buffer pH 6.0	0.0091
Buffer pH 7.0	0.0021
Buffer pH 8.0	0.152
Buffer pH 9.0	0.666
Buffer pH 10.0	1.03
Buffer pH 10.5	1.41
Buffer pH 11.0	2.03
Methanol	9.43
Dichloromethane	163.0
Acetone	162.4
Acetonitrile	92.7
Ethanol (95%)	3.6
Ethyl acetate	89.3

Since the Nimesulide-L-lysine salt exhibited increased aqueous solubility (5-7.5 mg/mL), Nimesulide-L-lysine-β or γ-cyclodextrin complexes were prepared by spray-drying [22, 31]. These complexes yielded remarkable aqueous solubilities. The incorporation of Nimesulide in a Nimesulide-L-lysine-β-cyclodextrin complex increased the water solubility by a factor of 10 at pH 1.5, and 160 at pH 6.8. A 3600-fold increase in solubility was reported in purified water (36.40 mg/mL for the complex, as compared to 0.01 mg/mL for Nimesulide alone) [22].

Solid dispersions of Nimesulide in polyvinyl pyrrolidone, polyethylene glycol, dextrin, and pregelatinized starch have been prepared and evaluated for dissolution rates [32, 33].

A water-soluble adduct of Nimesulide with an amino-sugar, *N*-methyl-glucamine, has been patented for injectable use [34]. Nimesulide micronized salts with metals such as sodium, potassium, calcium, magnesium, and zinc having improved bioavailability and pharmacokinetics have been prepared [35]. A Nimesulide choline salt has also been prepared [36].

The solubility of Nimesulide in supercritical carbon dioxide, measured using a dynamic saturation technique of pressures between 100 bar and 220 bar and at two temperatures, 312.5 K and 331.5 K, has been reported [37].

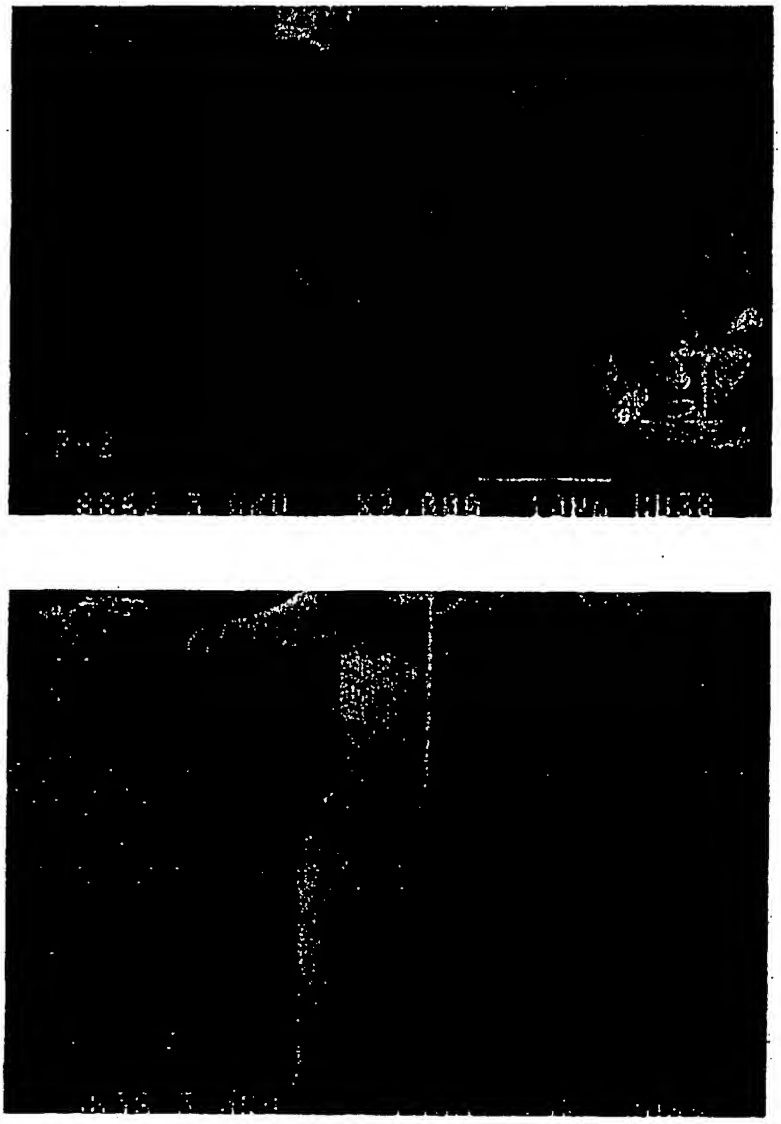
3.3 Partition Coefficient

The octanol/water partition coefficient of Nimesulide is 238, corresponding to a log P value of 2.376 [38]. A value of this magnitude clearly demonstrates the lipophilic character of the drug.

3.4 Particle Morphology

It is reported [2] that light-tan crystals are obtained from ethanol on crystallization. A commercial sample crystallized from ethanol yielded needle-shaped crystals [18]. The two samples were evaluated using electron microscopy, with the data being obtained on a JEOL JSM-6100 system (Figures 1a and 1b) [18]. As evident in the photomicrograph, the majority of the crystals exhibited a rod-like morphology.

Figure 1. Scanning electron photomicrographs obtained at a magnification of 2000X for (a) a commercial sample of Nimesulide, and (b) a recrystallization of the commercial sample.



Most of the crystals of the commercial sample were approximately 5 to 20 μm in length and 1 to 5 μm in width, whereas recrystallized commercial samples were more than 100 μm in length and 10-12 μm in width. One concludes from consideration of Figure 1 that the commercial sample is micronized before being used to produce dosage forms.

3.5 Crystallographic Properties

3.5.1 Single Crystal Structure

A full single crystal structural determination of Nimesulide has been reported [19]. The compound crystallizes in the monoclinic space group $C2/c$, with $a = 33.657(3)$ Å, $b = 5.1305(3)$ Å, $c = 16.0816(10)$ Å, and $\beta = 92.368\ (8)°$. In addition, $Z = 8$, $d_c = 1.476$; $K(F^2) = 0.0401$, $R_w(F^2) = 0.1146$ for 1908 reflections. The molecular conformation is stabilized by an intramolecular N-H···O hydrogen bond. The angle between the two phenyl rings is 74.7°. The cohesion of the crystal is the result of N-H···O intermolecular hydrogen bonds and van der Waals interactions. Figure 2 gives the structure derived from the data.

There is no definite information that conclusively demonstrates the existence of crystal polymorphs. There is a report, however, indicating a faster dissolution rate for crystals prepared by solvent change (1:1 ethanol / water) in the presence of 1% Tween 80 compared to those crystals in presence of polyethylene glycol 4000 and povidone K30 [20]. Adsorption of the surfactant or the water-soluble polymer (PEG or PVP) on the crystal surface could possibly account for any faster crystal dissolution.

3.5.2 X-Ray Powder Diffraction Pattern

The X-ray powder diffraction pattern of Nimesulide was obtained using a Philips PW1729 X-ray diffractometer system [18]. The radiation source was a copper (λ= 1.54820Å) high intensity X-ray tube operated at 35 kV and 20 mA.

Nimesulide was found to exhibit a strong and characteristic x-ray powder diffraction pattern, showing the crystalline nature of the powder. The powder pattern is found in Figure 3, and Table 2 provides a summary of scattering angles, d-spacings, and relative intensities.

Figure 2. Molecular conformation of Nimesulide.

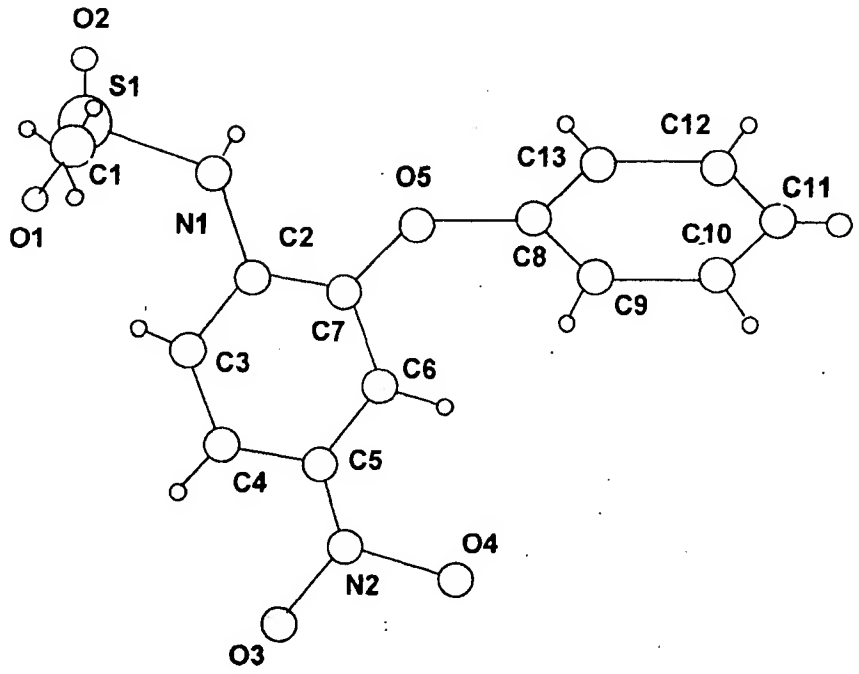


Figure 3. X-ray powder diffraction pattern of Nimesulide.

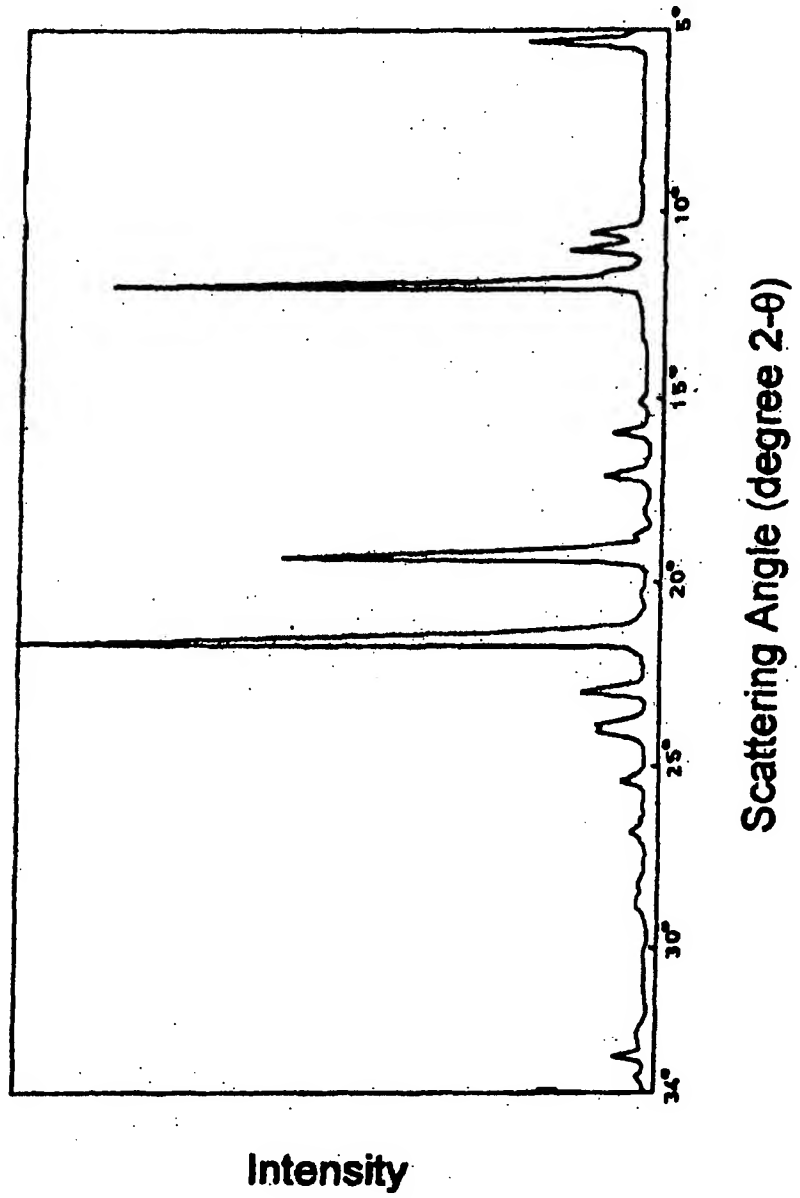


Table 2

Crystallographic Parameters Derived from the X-Ray Powder Pattern of Nimesulide

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
6.291	16.771	16.04
10.633	8.354	8.44
11.110	7.997	11.07
12.032	7.386	67.62
15.139	5.876	2.46
15.989	5.565	5.84
17.157	5.189	6.60
18.145	4.909	1.96
18.829	4.732	2.81
19.349	4.606	37.19
20.383	4.374	1.98
21.646	4.122	100.00
23.121	3.862	8.94
24.027	3.719	7.05
25.500	3.507	4.09
26.145	3.422	1.64
26.579	3.367	1.83
26.862	3.332	3.08
28.302	3.166	1.92
28.916	3.198	2.06
29.009	3.090	1.95
30.274	2.964	1.21

Although the powder pattern contains a number of scattering lines, the pattern is dominated by the intense scattering peaks located at 12.03, 19.34, and 21.6 degrees 2-θ.

3.6 Hygroscopicity

When exposed to relative humidities ranging from 60% to 70% (at an equilibrium temperature ranging from 25°C to 40°C), Nimesulide did not exhibit any measurable moisture pickup in over seven days. The compound is therefore determined to be non-hygroscopic.

3.7 Thermal Methods of analysis

3.7.1 Melting Behavior

The melting range of Nimesulide is 148 – 150°C, and the substance appears to melt without decomposition. Other reported values in the literature for melting range are 143 – 144.5°C [2] and 149°C [21].

3.7.2 Differential Scanning Calorimetry

The differential scanning calorimetry thermogram of Nimesulide was obtained using a Mettler-Toledo model DSC821, calibrated using 99.999% indium [18]. The thermogram shown in Figure 4 was obtained using a heating rate of 5°C/min, under an atmosphere of nitrogen. A single sharp endotherm was observed, having an onset of 148.71°C, a maximum at 149°C, and an endset at 151.67°C. The endotherm is assigned to the melting of the compound, and is characterized by an enthalpy of fusion equal to 650 Joule/g (200.4 kJ/mol). The quality of the thermogram indicates that DSC could be used as one of the techniques to determine the purity of Nimesulide.

3.7.3 Thermogravimetric Analysis

As an anhydrous material, Nimesulide exhibits no loss in weight until heated beyond the temperature of its thermal decomposition (approximately 300°C). The TG thermogram is shown in Figure 5 [18].

Figure 4. Differential scanning calorimetry thermogram of Nimesulide.

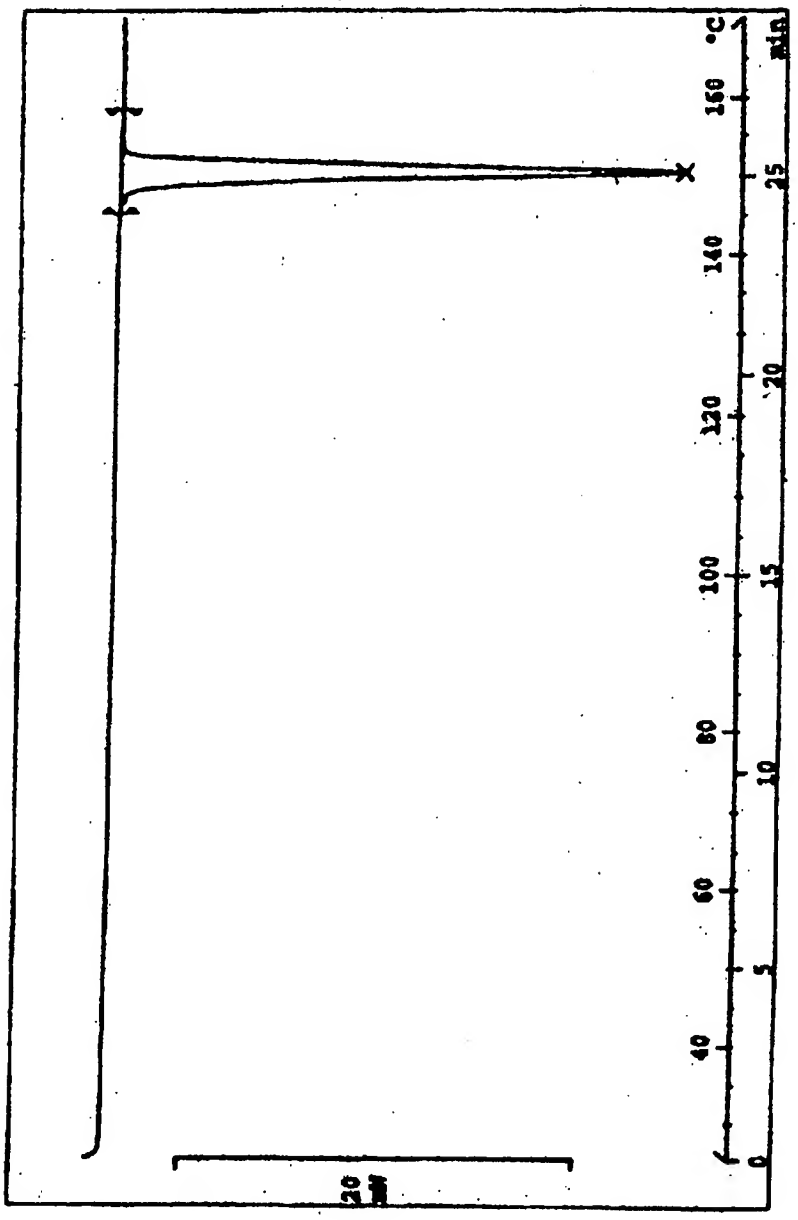
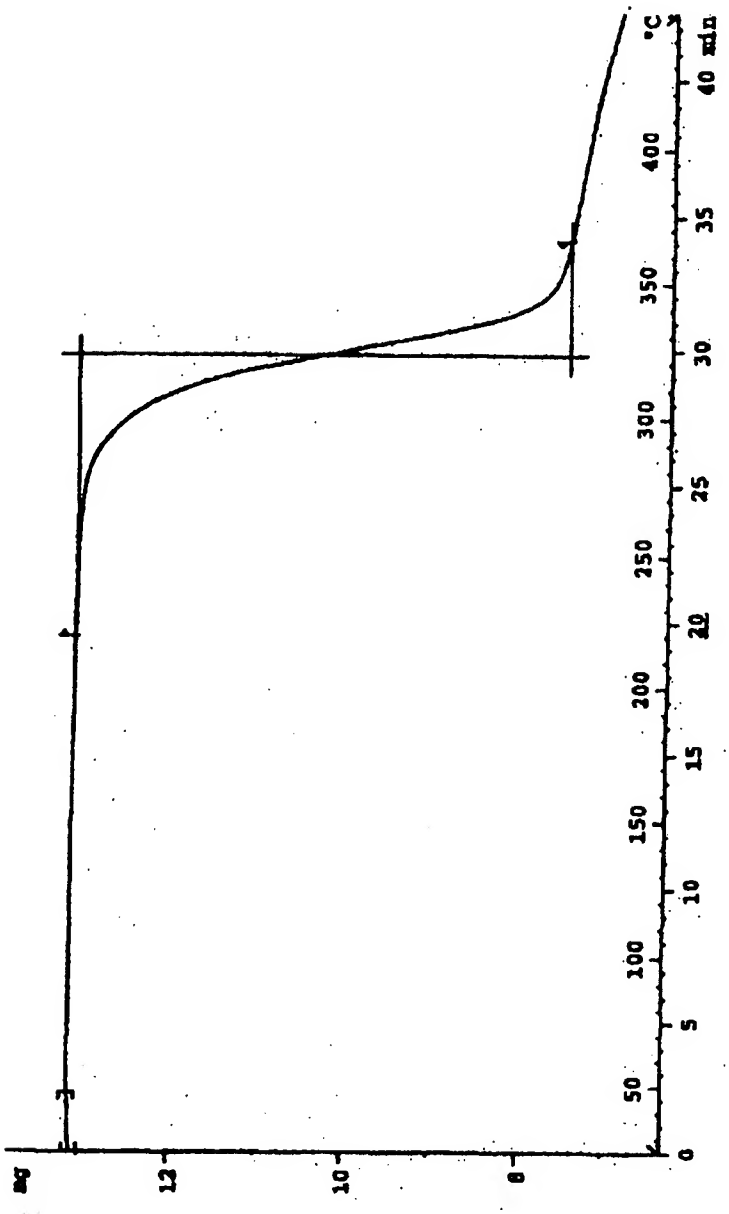


Figure 4. Thermogravimetric analysis thermogram of Nimesulide.



3.8 Spectroscopy

3.8.1 UV/VIS Spectroscopy

The ultraviolet spectrum of Nimesulide was recorded using a UV-Beckman DU 640i spectrophotometer, at a solute concentration of 0.04 mg/mL in methanol. The spectrum shown in Figure 6 exhibits maxima at 213, 296, and 323 nm [18].

3.8.2 Vibrational Spectroscopy

The infrared spectrum of Nimesulide obtained in a KBr pellet, and was recorded on a-Nicolet-Impact-400) FTIR spectrometer. The spectrum shown in Figure 7 exhibits principal stretching modes at 1593 cm^{-1} (aromatic rings), 1153 cm^{-1} (SO_2 antisymmetric stretch), 1342 cm^{-1} and 1520 cm^{-1} (aryl nitro group stretching), 1247 cm^{-1} (diaryl C-O stretching), and 3288 cm^{-1} (NH stretch) [18].

3.8.3 Nuclear Magnetic Resonance Spectrometry

3.8.3.1 ^{1}H-NMR Spectrum

The ^{1}H-NMR spectra of Nimesulide were recorded on a Bruker AC300F NMR spectrometer at 300 MHz, using deutero-chloroform as solvent and tetramethylsilane as the internal standard [18]. The spectra are shown in Figures 8 and 9, and Table 3 provides a summary of the proton assignments.

3.8.3.2 ^{13}C-NMR Spectrum

The ^{13}C-NMR spectrum (Figure 10) of Nimesulide was obtained in deutero-chloroform at ambient temperature using tetramethylsilane as the an internal standard [18]. The one-dimensional and the DEPT 135 (Figure 11), DEPT 90 (Figure 12), COSY 45 (Figure 13), and INVBTP (HETEROCOSY) (Figure 14) spectra were used to develop the ^{13}C chemical shift assignments that are summarized in Table 4.

Figure 6. Ultraviolet spectrum of Nimesulide in methanol.

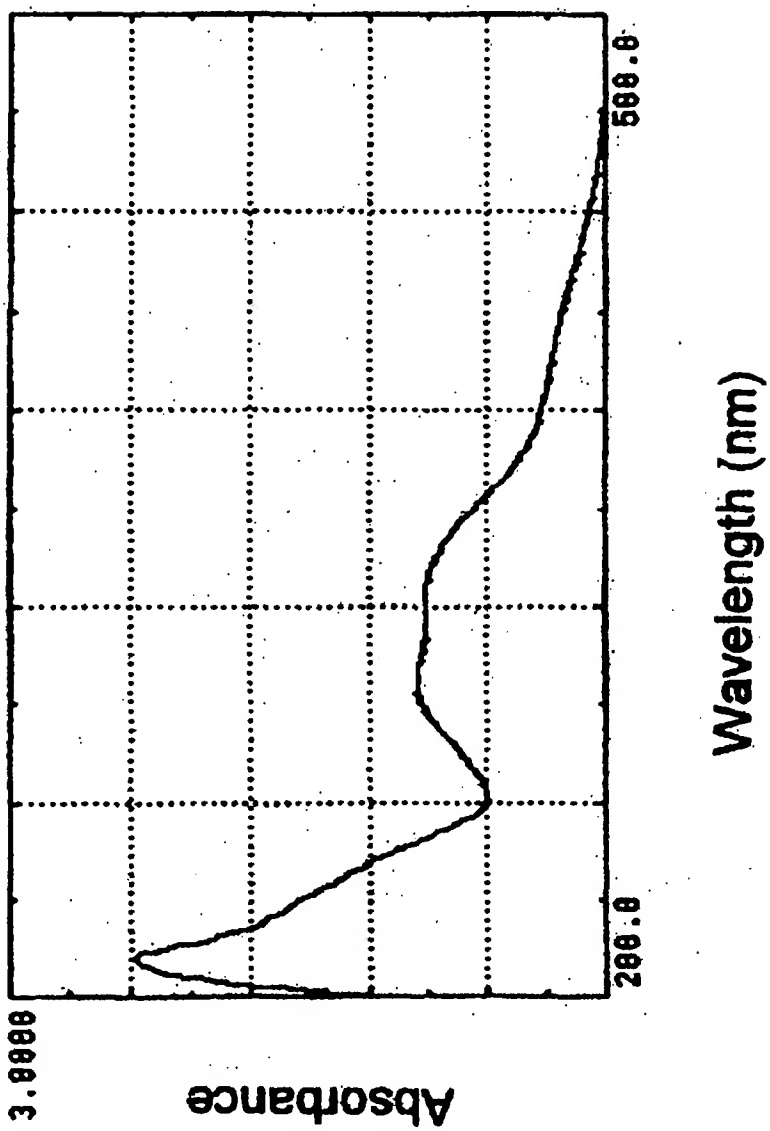


Figure 7. Infrared absorption spectrum of Nimesulide in a KBr pellet.

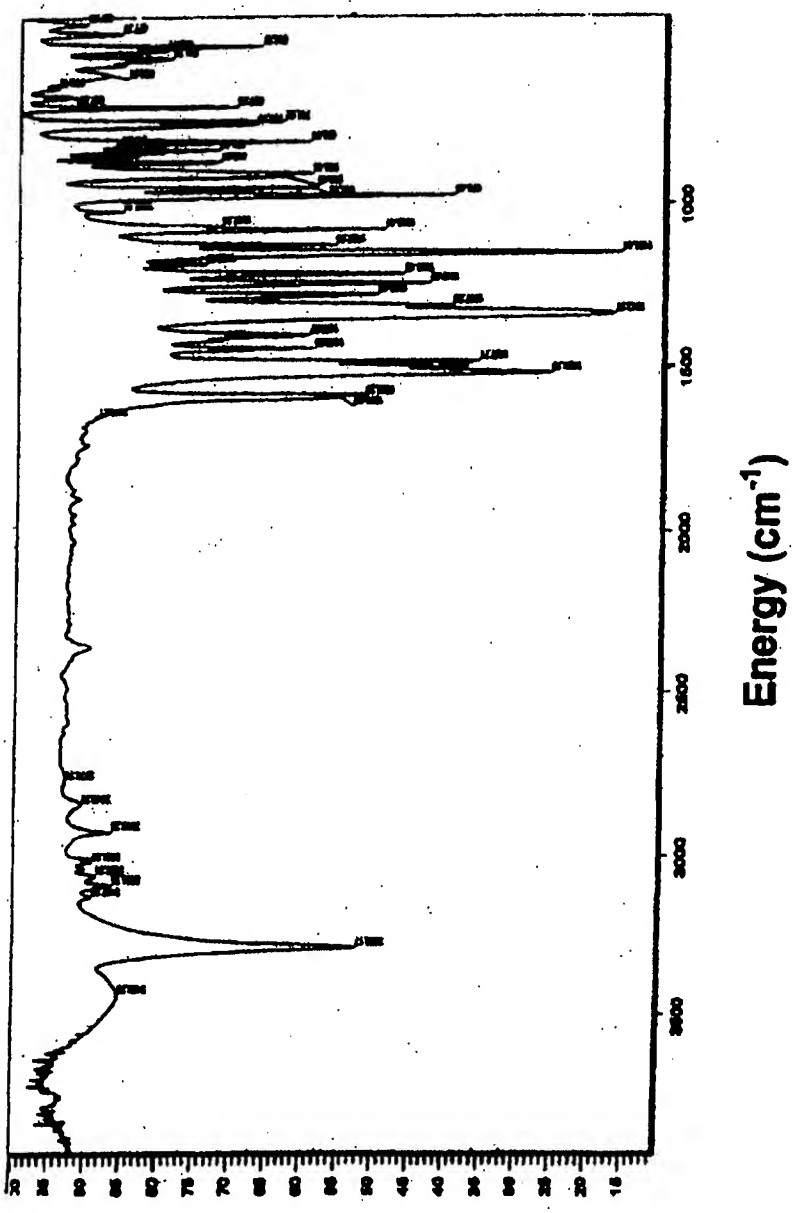


Figure 8. ^{1}H-NMR spectrum of Nimesulide in $CDCl_3$.

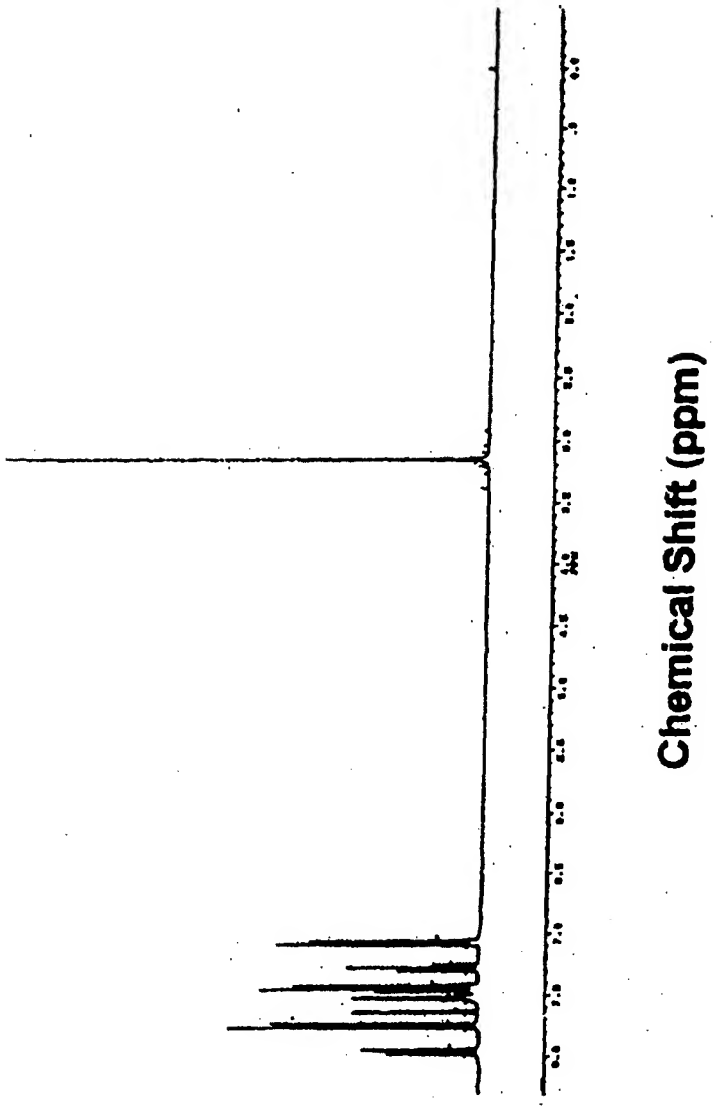


Figure 9. Expanded ^{1}H-NMR spectrum of Nimesulide in $CDCl_3$.

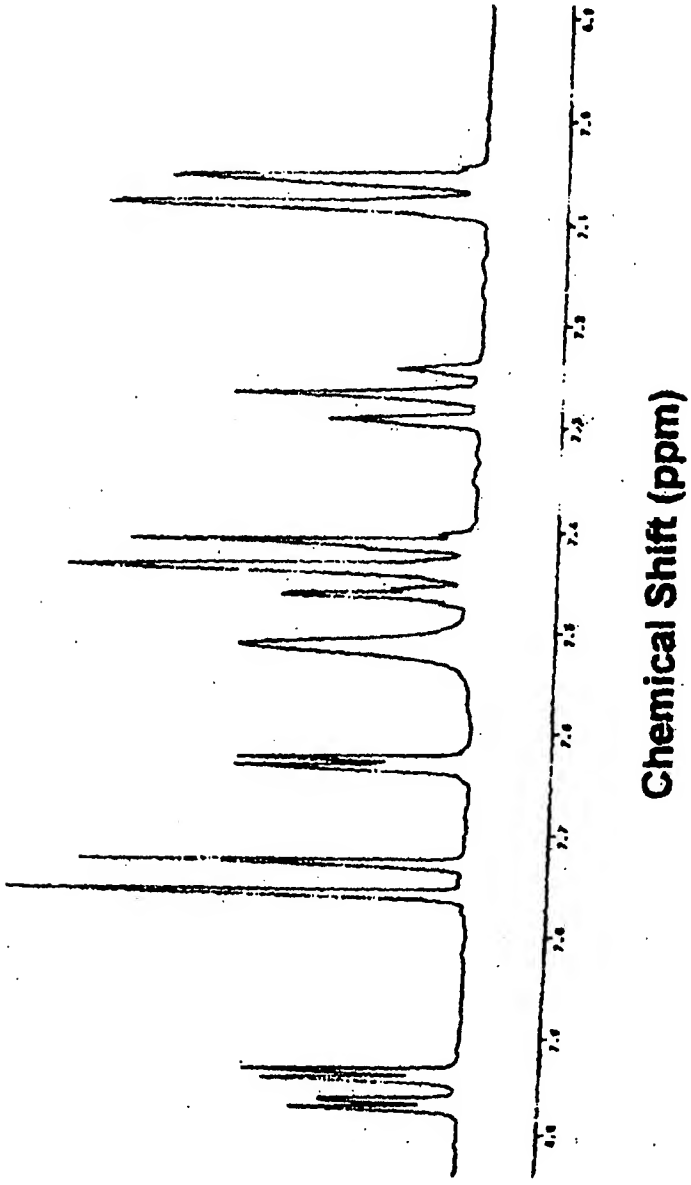
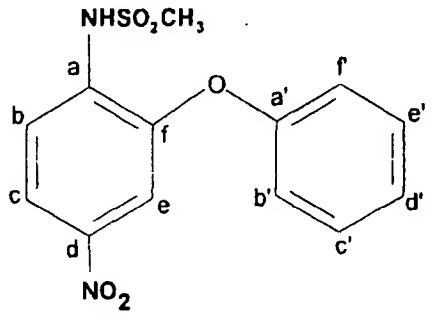


Table 3

Assignments for the Resonance Bands Observed in the ^{1}H-NMR Spectrum of Nimesulide



Chemical Shift (ppm)	Number of Protons	Multiplicity	Assignment
3.14	3	Singlet	$-SO_2CH_3$
7.05-7.08	2	Doublet	b';f'; Ar-H
7.24-7.29	1	Triplet	d'; Ar-H
7.41-7.46	2	Triplet	e'; c'; Ar-H
7.51	1	Singlet	$-NH-SO_2-$
7.61	1	Doublet	e; Ar-H
7.73-7.75	1	Doublet	b; Ar-H
7.93-7.97	1	Double-doublet	c; Ar-H

Figure 10. ^{13}C-NMR spectrum of Nimesulide in $CDCl_3$.

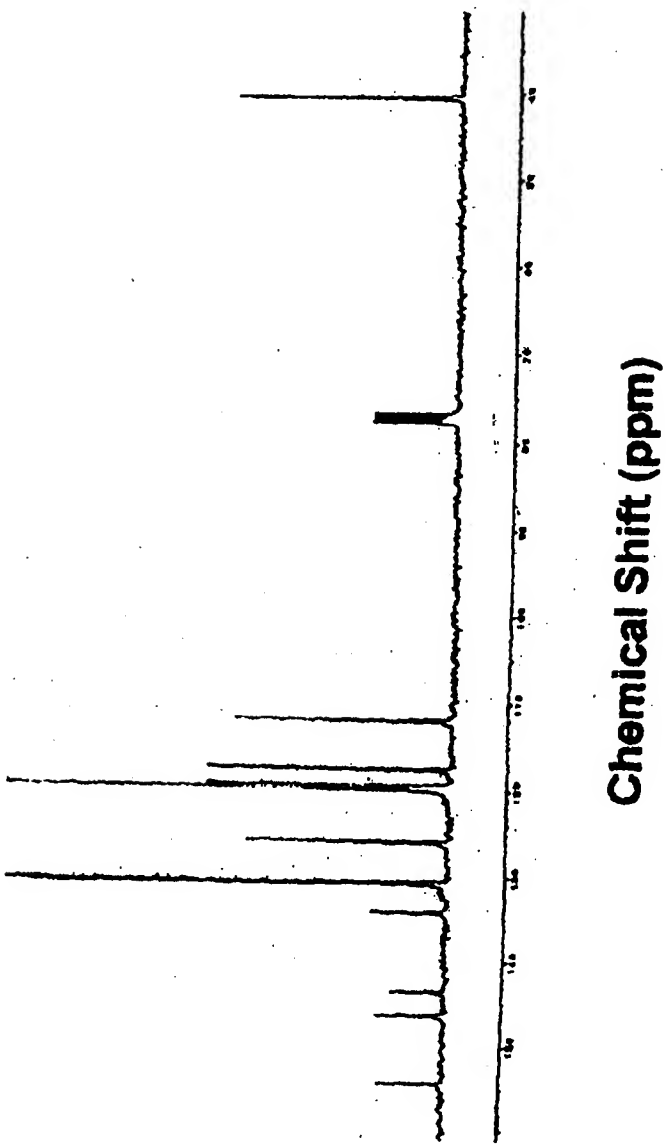


Figure 11. DEPT 135 ^{13}C-NMR spectrum of Nimesulide.

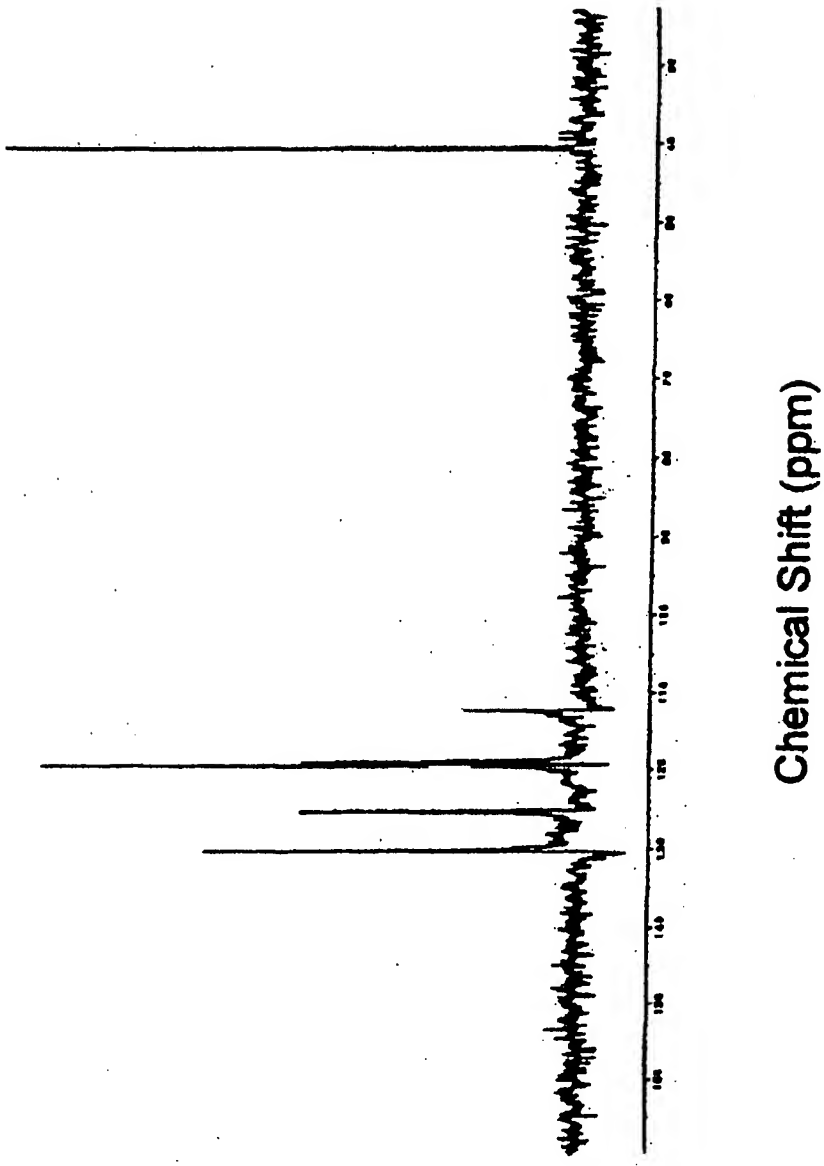


Figure 12. DEPT 90 ^{13}C-NMR spectrum of Nimesulide.

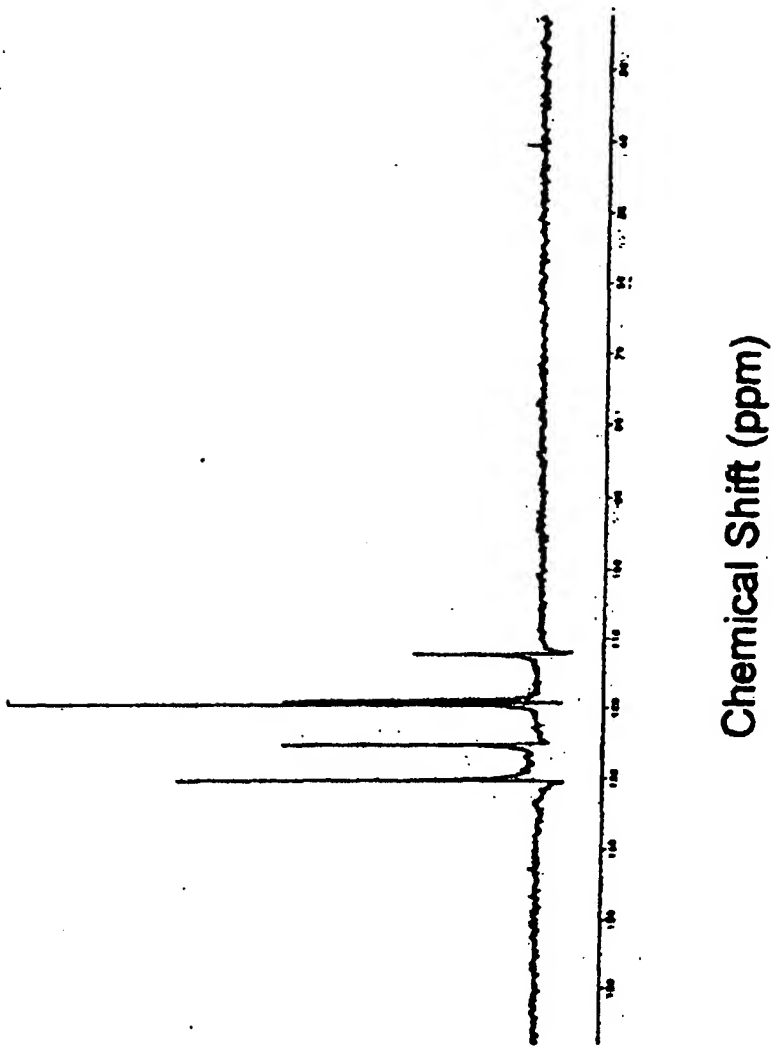


Figure 13. HOMOCOSY NMR spectrum of Nimesulide.

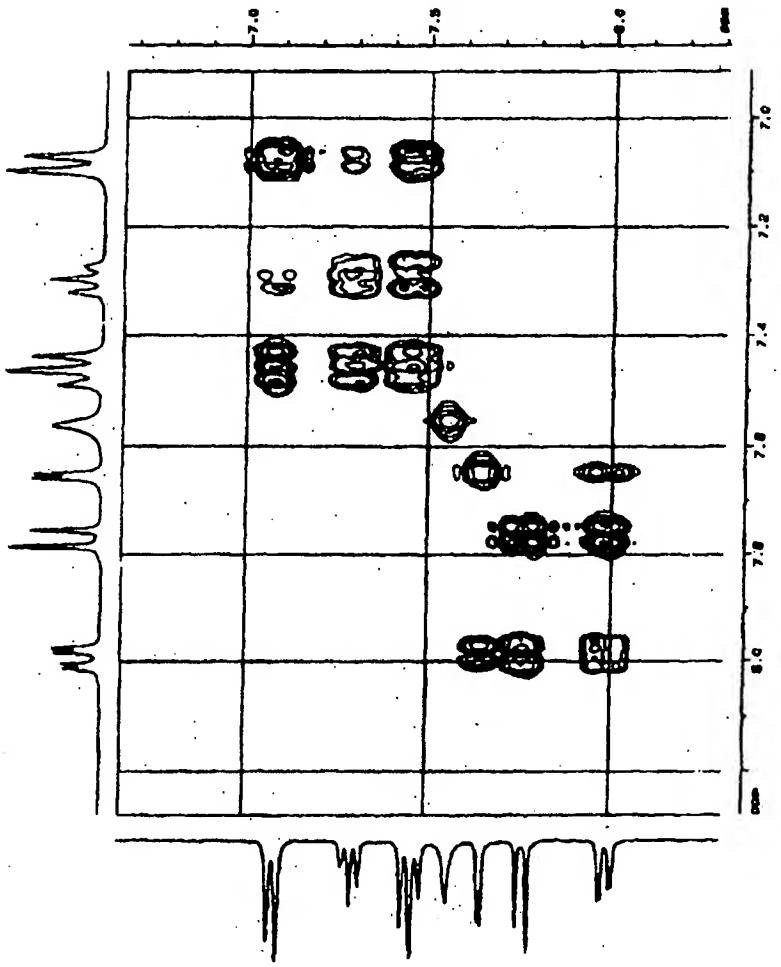


Figure 14. HETEROCOSY NMR spectrum of Nimesulide.

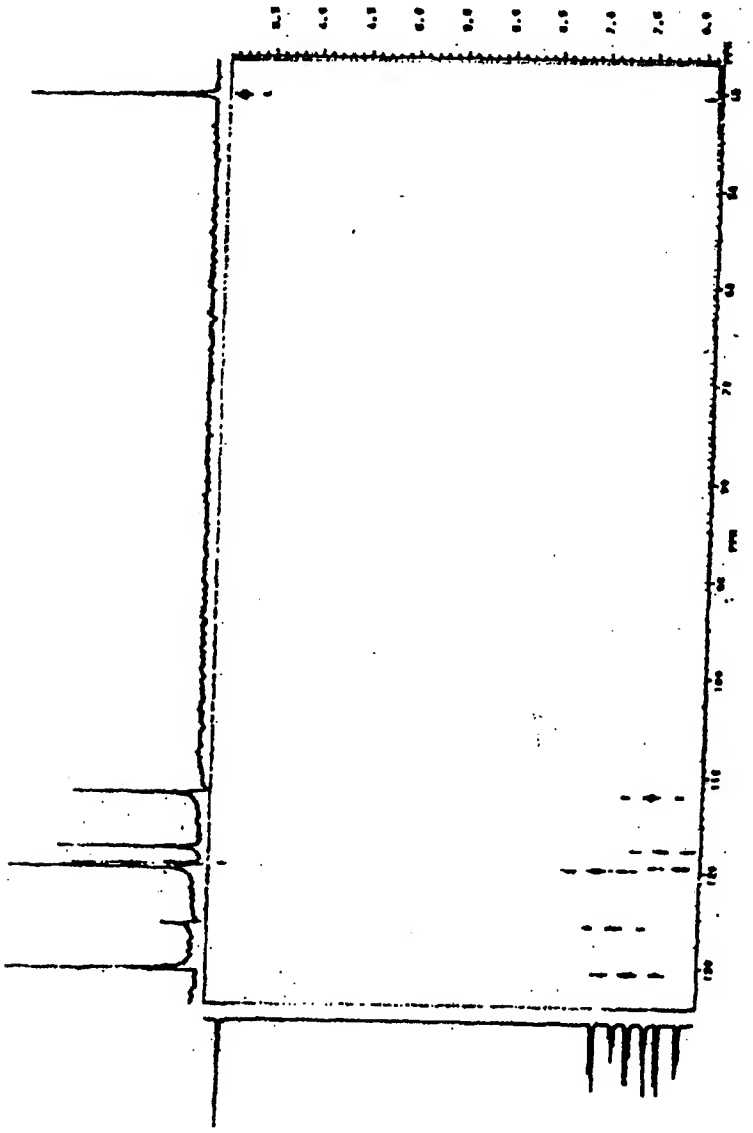
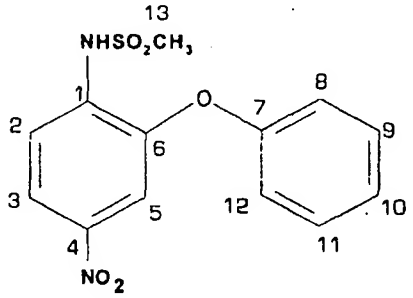


Table 4

Assignments for the Resonance Bands Observed in the ^{13}C-NMR Spectrum of Nimesulide



Chemical Shift (ppm)	Carbon Number
134.08	1
117.50	2
119.33	3
154.20	4
111.89	5
146.38	6
143.59	7
119.66	8,12
130.58	9,11
125.79	10
40.46	13

3.9 Mass Spectrometry

The mass spectrum of Nimesulide was recorded using a VG 70-250S mass spectrometer, and is shown in Figure 15 [18]. The molecular ion peak (M^+) was found at m/z = 308 (80%). The other characteristic peaks appeared at:

m/z = 229 (100%), due to $M^+ - SO_2CH_3$
m/z = 183 (37.4%), due to $M^+ - (SO_2 CH_3 - NO_2)$
m/z = at 154 (99.2%)
m/z = 77 (47.2%), due to $C_6H_5^+$

4. Methods of Analysis

4.1 Identification

The identification of Nimesulide can be made on the basis of its characteristic ultraviolet absorbance, or on the basis of its infrared absorption spectrum. A method for identification based on thin layer chromatography will be discussed in a later section.

4.2 Titrimetric Analysis

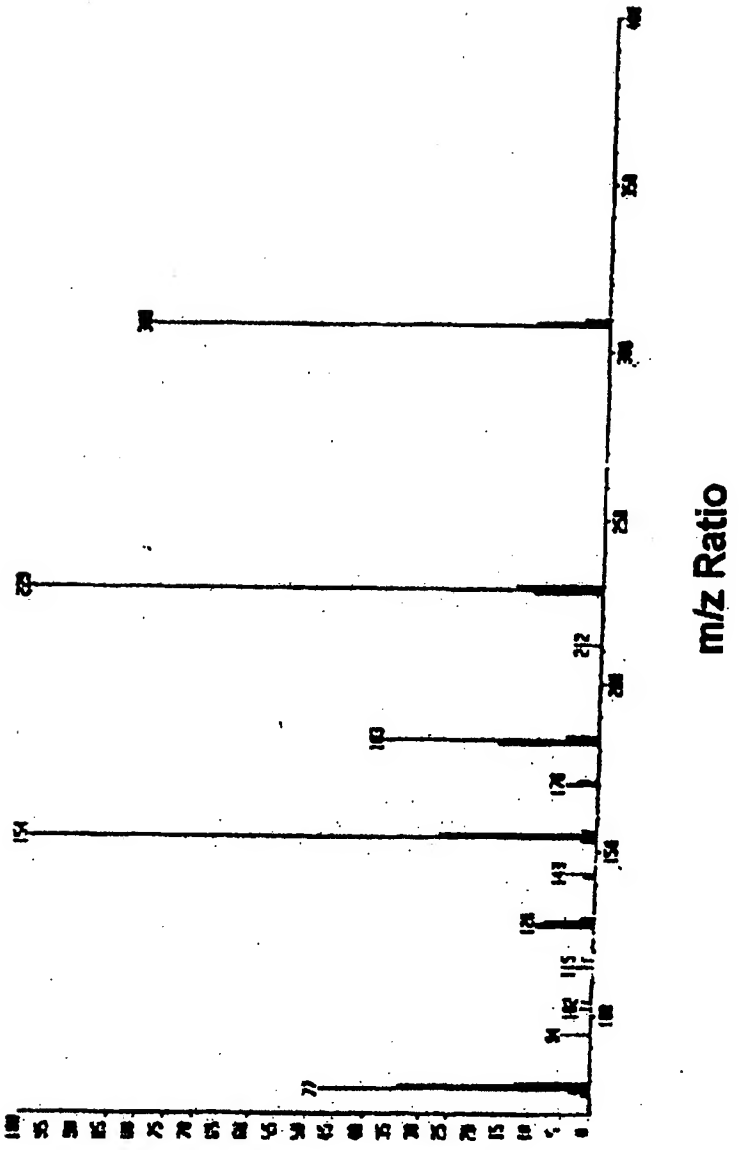
A titrimetric method has been reported for the assay of Nimesulide [3]. 0.240 g of sample is dissolved in 30 mL of acetone, to which 20 mL of water is added. The solution is titrated with 0.1M sodium hydroxide, and the end point is determined potentiometrically. Each milliliter of 0.1 M NaOH is equivalent to 30.83 mg of Nimesulide.

Potentiometric titrations have also been employed for the pKa determination of Nimesulide in methanol-water mixtures [39].

4.3 Electrochemical Analysis

Several electrochemical methods for the analysis of Nimesulide have been reported [43-45]. Nimesulide in hydro-alcoholic solution presents a cathodic response over a wide range of pH values (2-12), both by differential pulse and polarographic techniques. The results show only one well-defined peak or wave over all of the pH range studied. This wave corresponds to reduction of the nitro group at position 4.

Figure 15. Mass spectrum of Nimesulide.



The voltammetric oxidation shows one well-resolved signal within the pH range studied. This anodic signal could be attributed to the menthanesulfonamide group oxidation. For analytical purposes, a very well resolved diffusion controlled differential pulse polarographic peak obtained at pH 7.0 was selected. This peak was used to develop a new method for the determination of Nimesulide in pharmaceutical dosage forms. The recovery was 104.8%, with a RSD of 1.3%. The method was reported to be sufficiently accurate and precise so as to be applied in the individual tablet assay of commercial samples [43].

A simple D.C. polarographic method has been developed for the analysis of nitro-containing drugs (such as Nimesulide) in tablets. The optimum pH range for obtaining well resolved waves suitable for quantitative determination of the drug was found to be between 4.0 and 6.0. Both the standard addition and calibration methods were employed [45].

4.4 Spectroscopic Analysis

4.4.1 Fluorimetry

Nimesulide was determined by a fluorimetric method based on quenching the natural fluorescence of *N*-(1-naphthyl)ethylenediamine by reaction with the diazotized reduction product of Nimesulide [46]. In a methanolic solution, the drug was reduced by zinc dust in 1M hydrochloric acid and diazotized with sodium nitrite and hydrochloric acid. After the addition of sodium sulfamate and *N*-(1-naphthyl)ethylenediamine, the fluorescence was measured at 309/427 nm (excitation/emission). Beer's law was obeyed over the concentration range of 0.55 - 2.75 μg/mL, and the analyte recovery was 99.5 - 100.4%. Common tablet excipients did not interfere in the assay [46].

4.4.2 Colorimetry and Spectrophotometry

Several spectrophotometric methods have been reported for the determination of Nimesulide in pharmaceutical dosage forms [47-56]. Fallavana *et al.* [47] have reported an ultraviolet spectrophotometric method, based on the absorbance at 295 nm, for the determination of Nimesulide in tablets. The concentration of the working curve was 9.6 - 30.4 mg/mL, and Pearson's coefficient was 0.99978.

A spectrophotometric method has been described which is based on reduction of the nitro group of Nimesulide by zinc dust and hydrochloric acid, followed by diazotization and coupling with *N*-(1-naphthyl)ethtylene-diamine dihydrochloride, to form a stable purple chromophore absorbing at 557 nm [48, 50]. A simple spectrophotometric method based on formation of a blue species (λ_{max} = 600 nm) with Folin-Ciocalteu reagent has been described [51]. Methods based on the formation of a colored product from the reaction of Nimesulide with paradimethylamino-cinnamaldehyde (exhibiting maximum absorption at 525 nm), and on complex formation with 1,10-phenanthroline and Fe(III) (with maximum absorption at 490 nm) have been reported [52].

A rapid, accurate and simple method for determination of bulk Nimesulide, and in its dosage forms, based on the formation of a colored product with vanillin, has been reported [53]. Beer's law was obeyed over the range of 33.3 - 166.6 µg/mL, with an RSD of 0.38%. A bluish green chromophore was formed when reduced Nimesulide was reacted with 3-methyl-2-benzothiazolinonehydrazone hydrochloride in the presence of ferric chloride(absorption maximum of 600 nm). This latter method was employed for the analysis of tablets and suspensions containing Nimesulide [54]. The recovery was 99 - 101%.

In another method [55], formation of a yellow solution having maximum absorbance at 436 nm, was obtained on dissolving Nimesulide in 0.1 N sodium hydroxide. This was the basis for determination of the drug in pharmaceutical dosage formulations. Manna *et al.* [56] have reported formation of a greenish yellow chromophore in sodium hydroxide solution (pH 12) with Nimesulide, which exhibited a λ_{max} = 394 nm.

4.5 Bioassay

Bioassay techniques for non-steroidal anti-inflammatory drugs including Nimesulide have been reported [58-60]. When freshly drawn, heparinized human whole blood is incubated with 50 µM calcium ionophore A23187, and platelets are stimulated to produce thromboxane B_2 (Tx B_2) by activation of prostaglandin G/H synthase-1 (PGHS-1). Tx B_2 concentration, as measured by immunoassay, is maximal at 20 - 30 minutes and declines thereafter. Addition of non-steroidal anti-inflammatory drug prior to 30 minute stimulation with ionophore results in concentration dependent inhibition of Tx B_2 production [58]. *In-vivo*

assessment of Nimesulide cyclooxygenase-2 selectivity has been reported by Shah *et al.* [59]. An *ex vivo* assay to determine the cyclooxygenase selectivity of non-steroidal anti-inflammatory drugs including Nimesulide has been described [60].

4.6 Chromatographic Methods of Analysis

4.6.1 Thin Layer Chromatography

A test sample of Nimesulide complies to the identification test if the retention factor of the sample is identical to that of an authentic Nimesulide reference standard.

A thin layer chromatography method has been developed for Nimesulide which is based on the use of silica gel 60 GF_{254} (Merck) as the stationary phase, and 9:1:1 toluene / ether / ethyl acetate as the mobile phase. The sample is prepared by weighing 10 mg of drug substance, and dissolving in 1 mL of methanol. 5 µL of this solution is applied to the plate and dried. The plate is allowed to run to a height of approximately 12 cm, and then allowed to dry. The spot is viewed using short wave ultraviolet (254 nm), and the retention factor (R_f) for Nimesulide is 0.427. R_f values for 2-phenoxy-phenylmethanesulfonanilide (a process impurity) and for 4-amino-2-phenoxymethanesulfonanilide (the reduced product of Nimesulide) are 0.558 and 0.175 respectively [18].

4.6.2 Gas Chromatography

A gas chromatographic method, using a 10% SE-30 column, has been reported for the determination of Nimesulide in its pharmaceutical preparations [61].

4.6.3 High Performance Liquid Chromatography

Several high performance liquid chromatography (HPLC) systems for the determination of Nimesulide have been reported [62-69]. The details are summarized in Table 5

Table 5

HPLC Methods for the Determination of Nimesulide

Column	Mobile phase	Flow rate (mL/min)	Detection	Ref.
C18 reversed-phase column (0.46 x 30 cm; 10 µm particle size)	0.05 M phosphate buffer (pH 5.0) – methanol (50 : 50)	1	UV at 230 nm	62
µ-Bondapack C18 reversed - phase column	0.05 M phosphate buffer (pH 7.0)-methanol (45 : 55)	1	UV at 230 nm	63
ODS column	Water (1g/L Na_2HPO_4) – methanol (30 :70)	1	UV at 254 nm	64
µ Bondapack/µ Porasil C18 column	Methanol-0.05 M phosphate buffer (pH 3)	1	Electro chemical	65
Hypersil ODS2 column	Methanol-water-acetic acid (60:40:1)	1	UV at 230 nm	66
Supelcosil LC-18 DB reversed-phase column	0.05 M phosphate buffer (pH 3)-acetonitrile	1	UV at 230	57
Shandon Hypersil BDS C18 column (5 µm particle size; 250 x 4.6 mm)	Methanol-citrate (0.08 M)-phosphate (0.04M) buffer (pH 3) (68 : 32)	1	240	67
Ultrasphere OSD column (250 x 4.6 mm)	Methanol-water (70:30)		298	68
C18 reversed-phase column	Phosphate buffer (pH 5.5)-methanol-acetonitrile (50 : 20 : 30)	1.4	230	69

4.6.3.1 HPLC Determination in Biological Matrices

Sensitive and selective HPLC methods that quantitate Nimesulide and its major metabolites in a variety of biological matrices have been reported. The matrices include human plasma [62, 66, 67, 69] and urine [57, 62].

4.6.3.2 HPLC Determination in Dosage Forms

A HPLC method useful for the separation and quantification of Nimesulide and its related compounds in drug raw material has been reported [63]. HPLC determination of Nimesulide in tablets by electrochemical detection has been described, with a mean recovery 101.07% and RSD equal to 1.44% [65]. Nimesulide has been determined in gels using this technique, with a mean recovery of 100.1% and RSD equal to 1.3% [68].

4.6.4 High Performance Thin-Layer Chromatography

High performance thin-layer chromatography (HPTLC) has been found to be useful for the determination of Nimesulide in biological matrices and in dosage forms.

4.6.4.1 HPTLC Determination in Biological Matrices

A rapid and sensitive HPTLC assay has been developed for the measurement of Nimesulide in human plasma, and has proved to be useful for pharmacokinetic studies [4]. The method includes a single-stage extraction procedure without the use of an internal standard. Known amounts of extract and Nimesulide (100 and 200 ng, as external standard) were spotted on a pre-coated silica gel 60 F_{254} plates by a Camag Linomat IV autosampler. Quantification was achieved using a Camag TLC scanner-3. The recovery of the method was 97%.

A second method involves use of a reversed- phase HPTLC on C18 bonded plates and has been reported to be a useful alternative to normal-phase TLC separations on silica gel [70].

4.6.4.2 HPTLC Determination in Dosage Forms

A simple, fast and accurate HPTLC method has been described for the determination of Nimesulide in pharmaceutical preparations [71]. Triclosan was used as an internal standard. The analysis involves use of Merck silica gel plates 60 F_{254}, a mobile phase of 9:1 chloroform / toluene, and a scanner detection wavelength of 300 nm. The average recovery was reported to be within pharmacopoeial limits.

Another method reported [72] was based on the use of toluene-methanol (8:2) as the mobile phase and densitometry at 324 nm. Plots of peak area against the drug concentration were linear over the range of 20 - 200 ng/μL. The recovery was close to 100%.

4.7 Capillary Zone Electrophoresis

Capillary zone electrophoresis has been described as one of the methods for the determination of pKa of non-steroidal anti-inflammatory drugs which are sparingly soluble in aqueous solutions. The methodology relies on the measurement of the effective mobility of ionic species in electrophoretic buffers prepared at different pH values [42].

5. Stability

The stability of Nimesulide and its main metabolite (hydroxy-Nimesulide) has been reported in plasma [21]. The study indicated that blank plasma samples were spiked at 50 and 2500 ng/mL with Nimesulide and hydroxy-Nimesulide and stored frozen at -20° C. After ten months five samples/concentrations were thawed and analyzed in parallel with freshly spiked plasma samples. The respective recoveries found at two concentrations were 102.0 and 103.0% for Nimesulide and 100.0 and 101.0% for hydroxy-Nimesulide, confirming that no degradation of either drug occurred.

6. Metabolism

Nimesulide is mainly eliminated by metabolic transformation, with the major metabolite being 4-hydroxy-Nimesulide, or 4-nitro-2-(4′-hydroxy-phenoxy)methanesulfonanilide (compound **a** in Figure 16) [70, 73, 74]. Minor metabolites have also been detected. A unique metabolite of Nimesulide characterized as 4-amino-2-phenoxymethanesulfonanilide (compound **b**, Figure 16) resulting from reduction of the nitro group, has been reported in equine blood and urine samples of [75].

Recently Carini *et al.* [57] have characterized and quantitatively determined the main urinary metabolites (Figure 16) of Nimesulide in man following a single oral administration of 200 mg. These were found resulting from hydroxylation of the phenoxy nucleus (a), reduction of the nitro group (b), concomitant hydroxylation and reduction (c) and *N*-acetylation of b (d) and c (e) metabolites. The bulk of the metabolites were in conjugated form, and accounted for approximately 40% of the administered dose.

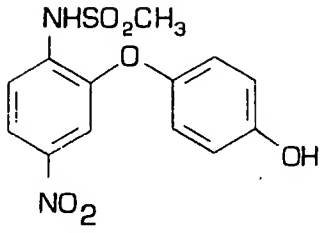
Electron impact ionization mass spectrometry was employed to elucidate the structures of the metabolites of Nimesulide [57].

7. Pharmacokinetics and Bioavailability

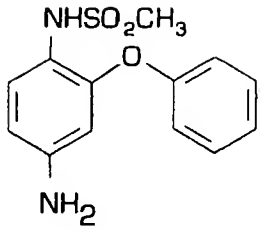
7.1 Pharmacokinetics

The pharmacokinetics of Nimesulide has been extensively investigated [76-84]. Studies have been carried out following oral or rectal administration in healthy volunteers, pediatric patients, patients with predisposition for altered pharmacokinetics, and in the elderly [74]. Bernareggi [81] has carried out a detailed clinical pharmacokinetic study of Nimesulide, and has reported that oral administration of Nimesulide tablet, granule, or suspension form in healthy human volunteers resulted in a rapid and extensive absorption of the drug. The mean peak concentration of 2.86 to 6.50 mg/L was reached within 1.22 to 2.75 hours of administration. The presence of food did not reduce either the rate or extent of absorption. Nimesulide is rapidly distributed and has an apparent volume of distribution ranging between 0.18 and 0.39 L/kg. The established mean terminal elimination half-life varied from 1.80 to 4.73 hours.

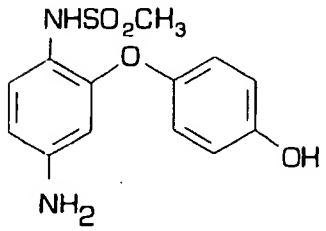
Figure 16. Metabolites of Nimesulide.



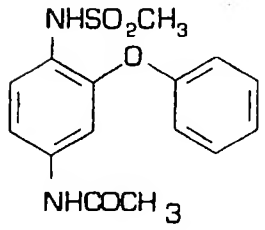
a



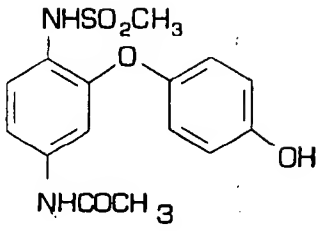
b



c



d



e

Excretion of the unchanged drug in urine and feces is reported to be negligible. It is largely eliminated via its metabolites 50.5 to 62.5% in the urine and 17.9 to 36.2% in feces.

The total plasma clearance of Nimesulide is reported [81] to be 31.02 to 106.16 mL/h/kg, reflecting almost exclusive metabolic clearance. The pharmacokinetic profile of the drug in children and the elderly is not different from that of young healthy individuals. However, hepatic insufficiency is reported to remarkably reduce the rate of elimination of the drug necessitating a dose reduction (4 to 5 times) in patients with hepatic impairment. Moderate renal failure does not alter the pharmacokinetic profile of the drug.

Pharmacokinetic interaction between Nimesulide and other drugs given in combination such as glibenclamide, cimetidine, furosemide, theophylline, warfarin, digoxin, and antacids were reported to be absent or of no apparent clinical relevance. In another study Gandini *et al.* [76] have carried out first dose and steady state pharmacokinetics of Nimesulide and its 4-hydroxy metabolite in healthy volunteers. After a single dose of 200 mg, peak plasma concentration of Nimesulide (9.85 μg/mL) were reached at 3.17 hours and the half-life during the elimination phase was 4.95 hours. Plasma concentration on the seventh day, predicted from the results of the first day, were similar to the measured values. The study pointed out that pharmacokinetics of Nimesulide or its metabolite after single or repeated dose were not time or dose dependent.

Sengupta *et al.* [80] studied the analgesic efficacy and pharmacokinetics of topical Nimesulide gel in healthy human volunteers and carried out a double-blind comparison with piroxicam, diclofenac, and placebo. Nimesulide exhibited better efficacy than did diclofenac, piroxicam, and placebo. The superior analgesic activity of Nimesulide as gel formulation correlated with its pharmacokinetic profile. The study concluded that the topical route of administration may be a safe and effective alternative to the presently used oral or related routes [80].

Study of the pharmacokinetic profile of a parenteral formulation of Nimesulide demonstrated that Nimesulide intramuscularly administered may be superior to other routes of administration when fast onset of action is required [82]. A summarized version of the pharmacokinetic profile of Nimesulide in different dosage forms has been presented by Singla and

coworkers [74]. In a review, Rainsford [83] analyzed the relationship of Nimesulide safety to its pharmacokinetics and concluded that Nimesulide is associated with a relatively low occurrence of adverse drug reactions, especially in the gastrointestinal tract. The reactions in the liver are within or below the general incidence with other NSAIDs'.

7.2 Bioavailability

The bioavailability of Nimesulide has been studied in healthy volunteers [78, 79, 81, 85]. The relative bioavailability of domestic or imported Nimesulide was determined after giving a single oral dose of 200 mg to ten volunteers in randomized crossover study by Chinese scientists Li *et al.* [79]. The plasma concentration of Nimesulide was assayed by high performance liquid chromatography. The concentration time curve of Nimesulide conformed to a one-compartment model, and the main parameter of domestic Nimesulide were:

$T_{½KC}$ = 3.61 ± 1.43 hours
T peak = 2.07 ± 0.63 hours
C_{max} = 0.46 ± 2.06 mg/L
AUC = 76.39 ± 17.62 mg/L/ hours

The relative bioavailability of domestic tablets was 92.2%. The results of these factors, analysis of variance, and Bayesian method, showed the two formulations to be bioequivalent.

In another study, the bioavailability of Nimesulide tablets and granules were identical, as shown in a random crossover design in humans [85]. Bernareggi [81] has reported that when Nimesulide was administered in suppository form, the C_{max} was lower and occurred later than after oral administration. The bioavailability of Nimesulide via suppository administration ranged from 54 to 64% relative to that of orally administered formulations.

The bioequivalence of two new pharmaceutical formulations of Nimesulide (sachet and effervescent tablets) with 100 mg Nimesulide, was investigated in 12 healthy volunteers [86]. No significant differences were found in the rate of bioequivalence analysis (C_{max} and T_{max}) and extent of Nimesulide absorption in the test (effervescent tablets) and in the reference drug (sachets).

8. Protein Binding

Bree *et al.* [73] have carried out a detailed equilibrium dialysis study of the binding of Nimesulide within human serum to isolated proteins and to erythrocytes. Within the range of therapeutic concentrations, Nimesulide was 99% bound to serum involving a non-saturated process ($NK_A = 91$). This binding was almost identical to binding of Nimesulide to serum albumin ($NK_A = 95$). Binding of Nimesulide to serum albumin was not affected by physiological concentrations of free fatty acids. The retention of Nimesulide by erythrocytes suspended in buffer was moderate (67%), although in whole blood no erythrocyte binding was observed because of the greater affinity of this drug for serum. Over the range of therapeutic concentrations (2.5 to 63 μmol/L) the free fraction of Nimesulide in serum remains constant.

Serum binding was decreased in samples obtained from patients with renal failure or hepatic cirrhosis associated with hypoalbuminemia and hyperbilirubinemia, respectively. The binding of Nimesulide at therapeutic concentrations was unaffected by warfarin, cefoperazone, furosemide, glibenclamide, tamoxifen, or digitoxin. However, valproic acid [73], fenofibrate [73, 87] (80 μmol/L), salicylic acid [87], tolbutamide [87] may displace Nimesulide on concurrent administration. It was reported that the principal metabolite of Nimesulide 4-hydroxy-Nimesulide, significantly increased the free fraction of the drug. Although methotrexate had no effect on the free fraction of Nimesulide, the free fraction of methotrexate was significantly increased in the presence of Nimesulide. It was also demonstrated by the study that there are two distinct Nimesulide binding sites, site I and site II, on serum albumin (10 μmol/L) with different affinities: site II $K_A = 3.57 \times 10^5$ L/mol and site I $K_A = 1.24 \times 10^5$ L/mol. It was indicated that Nimesulide binds to site II with higher affinity and to a lesser extent to site I. Bernareggi [81] has also reported that Nimesulide is extensively bound to albumin; the unbound fraction in plasma being 1%. The unbound fraction increased to 2 and 4% in patients with renal or hepatic insufficiency.

9. Toxicity

Nimesulide is the leading molecule of a new class of sulfonanilides among non-steroidal anti-inflammatory drugs that has shown a significant inhibitory selectivity towards cyclooxygenase-2 without affecting

cyclooxygenase-1. This results in equivalent efficacy against pain and inflammation but with a better safety profile [88]. Nimesulide appears to be particularly useful for patients who have allergic hypersensitivity to aspirin or NSAIDs [89]. Studies have suggested Nimesulide as an alternative treatment in NSAIDs intolerant patients [90, 91].

There are very few reports of toxicity or adverse effects of Nimesulide. Though well-documented cases of acute hepatitis have not yet been reported with this drug, there is one report [92] on six patients who developed acute liver damage after initiation of Nimesulide. From clinical and histological data, it appears that both immunological and metabolic idiosyncratic reactions can be invoked as pathogenic mechanism of Nimesulide-induced liver disease. Although thrombocytopenia is a common feature in patients infected with HIV, one group of workers considered that thrombocytopenia in one of their patients was related to the use of Nimesulide [93].

A study has provided evidence that atopy and history of allergic reactions to antimicrobial drugs increase the likelihood of intolerance of Nimesulide in subjects allergic to NSAID's [94]. Risk factors for Nimesulide intolerance in patients with NSAID-induced skin disorders have been investigated [95]. Drug interactions with Nimesulide have been reviewed [87].

10. Dosage and Pharmaceutical Formulations

The usual adult oral and rectal dosage of Nimesulide for the treatment of a wide variety of inflammatory and pain states are 100 and 200 mg twice daily [84]. Nimesulide suspension and granules were commonly administered at a dosage of 5 mg/kg/day divided in two or three daily doses in paediatric clinical trials. Nimesulide has been formulated into various pharmaceutical forms. They include tablets [77, 79, 85], effervescent tablets [86], granules [77, 85], sachet [86], suspensions [77, 96], emulsions [97, 98], injectables [99 - 101] and suppositories [77].

Three multiple w/o/w emulsions containing Nimesulide when compared with drug suspension showed a slow and controlled release of Nimesulide with prolonged anti-inflammatory activity in rats [97]. Effect of the nature

of oil, presence of electrolytes, phase volume ratios, and pH of the aqueous phase on the *in vitro* release of Nimesulide from multiple w/o/w emulsions was also studied [98]. Improved oral formulations for better drug delivery have been patented, such as compositions containing inclusion complexes for increased bioavailability [102], and granules for the formation of rapidly disintegrating orally soluble tablets [103]. These tablets are capable of disintegrating inside the mouth of the patient within a very short time.

A pharmaceutical preparation comprising coated capsules or tablets containing liposome powder encapsulating Nimesulide has been patented for improved oral bioavailability [104]. It is reported that blood levels of Nimesulide in volunteers was 7.31 μg/mL as compared with 2.69 μg/mL. A composition containing piperine, a bioavailability enhancer, has also been patented which is characterized in having clinically significant increased bioavailability when compared to the known formulations [105]. Recently, novel drug delivery approaches have been applied and Nimesulide has been formulated as solid dispersion [106], osmotic pumps [107], and transdermal delivery systems [108, 109].

A number of pharmaceutical preparations containing Nimesulide for topical use have been developed. These include gels [110-112], creams [113, 114], and liquid crystals [115]. A hydroalcoholic mouthwash containing Nimesulide has been developed for local use, with application to the oral and rhinopharyngeal cavity for the treatment of inflammation of oral and rhinopharyngeal mucosa [116].

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5. U.S. Patent No. 5,510,118 to Bosch et al.

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United States Patent [19]

Bosch et al.

[11] **Patent Number: 5,510,118**

[45] **Date of Patent: Apr. 23, 1996**

[54] **PROCESS FOR PREPARING THERAPEUTIC COMPOSITIONS CONTAINING NANOPARTICLES**

[75] Inventors: **H. William Bosch**, Bryn Mawr; **Donna M. Marcera**, Collegeville; **Ronald L. Mueller**, Downingtown; **Jon R. Swanson**, Macungie; **Dinesh S. Mishra**, Harleysville, all of Pa.

[73] Assignee: **NanoSystems LLC**, Collegeville, Pa.

[21] Appl. No.: **388,092**

[22] Filed: **Feb. 14, 1995**

[51] **Int. Cl.**[6] **A61K 9/14**

[52] **U.S. Cl.** **424/489**; 424/488; 424/490

[58] **Field of Search** 424/488, 489, 424/490, 22

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Primary Examiner—Thurman K. Page
Assistant Examiner—William E. Benston, Jr.
Attorney, Agent, or Firm—Rudman & Balogh

[57] **ABSTRACT**

A process of preparing nanoparticulate drug substances comprising the steps of: preparing a premix of the drug substance and a surface modifier, and subjecting the premix to mechanical means to reduce the particle size of the drug substance, the mechanical means producing shear, impact, cavitation and attrition.

20 Claims, No Drawings

PROCESS FOR PREPARING THERAPEUTIC COMPOSITIONS CONTAINING NANOPARTICLES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a process for preparing therapeutic compositions containing nanoparticles.

2. Reported Developments

Bioavailability is the degree to which a drug becomes available to the target tissue after administration. Many factors can affect bioavailability including the dosage form and various properties, e.g., dissolution rate of the drug. Poor bioavailability is a significant problem encountered in the development of pharmaceutical compositions, particularly those containing an active ingredient that is poorly soluble in water. Poorly water soluble drugs, i.e., those having a solubility less than about 10 mg/ml, tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation. Moreover, poorly water soluble drugs tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with fully soluble drug substances.

It is known that the rate of dissolution of a particulate drug can increase with increasing surface area, i.e., decreasing particle size. Consequently, methods of making finely divided drugs have been studied and efforts have been made to control the size and size range of drug particles in pharmaceutical compositions. For example, dry milling techniques have been used to reduce particle size and hence influence drug absorption. However, in conventional dry milling, as discussed by Lachman et al, *The Theory and Practice of Industrial Pharmacy,* Chapter 2, "Milling", p. 45, (1986), the limit of fineness is reached in the region of 100 microns (100,000 nm) when material cakes on the milling chamber. Lachman et al note that wet grinding is beneficial in further reducing particle size, but that flocculation restricts the lower particle size limit to approximately 10 microns (10,000 nm). However, there tends to be a bias in the pharmaceutical art against wet milling due to concerns associated with contamination. Commercial airjet milling techniques have provided particles ranging in average particle size from as low as about 1 to 50 μm (1,000–50,000 nm). However, such dry milling techniques can cause unacceptable levels of dust.

Other techniques for preparing pharmaceutical compositions include loading drugs into liposomes or polymers, e.g., during emulsion polymerization. However, such techniques have problems and limitations. For example, a lipid soluble drug is often required in preparing suitable liposomes. Further, unacceptably large amounts of the liposome or polymer are often required to prepare unit drug doses. Further still, techniques for preparing such pharmaceutical compositions tend to be complex. A principal technical difficulty encountered with emulsion polymerization is the removal of contaminants, such as unreacted monomer or initiator, which can be toxic, at the end of the manufacturing process.

U.S. Pat. No. 4,540,602 (Motoyama et al) discloses a solid drug pulverized in an aqueous solution of a water-soluble high molecular substance using a wet grinding machine. However, Motoyama et al teach that as a result of such wet grinding, the drug is formed into finely divided particles ranging from 0.5 μm (500 nm) or less to 5 μm (5,000 nm) in diameter.

EPO 275,796 describes the production of colloidally dispersible systems comprising a substance in the form of spherical particles smaller than 500 nm. However, the method involves a precipitation effected by mixing a solution of the substance and a miscible non-solvent for the substance and results in the formation of non-crystalline nanopanicles. Furthermore, precipitation techniques for preparing particles tend to provide particles contaminated with solvents. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical.

U.S. Pat. No. 4,107,288 describes particles in the size range from 10 to 1,000 nm containing a biologically or pharmacodynamically active material. However, the particles comprise a crosslinked matrix of macromolecules having the active material supported on or incorporated into the matrix.

U.S. Pat. No. 5,145,684 discloses a process for preparing particles consisting of a crystalline drug substance having a surface modifier or surface active agent adsorbed on the surface of the particles in an amount sufficient to maintain an average particle size of less than about 400 nanometers. The process of preparation comprises the steps of dispersing the drug substance in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the drug substance to an average particle size of less than 400 nm. The particles can be reduced in the presence of a surface active agent or, alternatively, the particles can be contacted with a surface active agent after attrition. The presence of the surface active agent prevents flocculation/agglomeration of the nanoparticles.

The mechanical means applied to reduce the particle size of the drug substance is a dispersion mill, the variety of which include a ball mill, an attrition mill, a vibratory mill and media mill, such as sand mill, and a bead mill.

The grinding media for the particle size reduction is spherical or particulate in form and includes: ZrO_2 stabilized with magnesia, zirconium silicate, glass, stainless steel, titania, alumina and ZrO_2 stabilized with yttrium. Processing time of the sample can be several days long. This patent is incorporated herein in its entirety by reference.

To a more limited extent the prior art also utilized microfluidizers for preparing small particle-size materials in general. Microfluidizers are relatively new devices operating on the submerged jet principle. In operating a microfluidizer to obtain nanoparticulates, a premix flow is forced by a high pressure pump through a so-called interaction chamber consisting of a system of channels in a ceramic block which split the premix into two streams. Precisely controlled shear, turbulent and cavitational forces are generated within the interaction chamber during microfluidization. The two streams are recombined at high velocity to produce shear. The so-obtained product can be recycled into the microfluidizer to obtain smaller and smaller particles.

The prior an has reported two distinct advantages of microfluidization over conventional milling processes (such as reported in U.S. Pat. No. 5,145,684, supra): substantial reduction of contamination of the final product, and the ease of production scaleup.

Numerous publications and patents were devoted to emulsions, liposomes and/or microencapsulated suspensions of various substances including drug substances produced by the use of microfluidizers. See, for example:

1) U.S. Pat. No. 5,342,609, directed to methods of preparing solid apatite particles used in magnetic resonance imaging, x-ray and ultrasound.

2) U.S. Pat. No. 5,228,905, directed to producing an oil-in-water dispersion for coating a porous substrate, such as wood.

3) U.S. Pat. No. 5,039,527 is drawn to a process of producing hexamethylmelamine containing parenteral emulsions.

4) G. Gregoriadis, H. Da Silva, and A. T. Florence, "A Procedure for the Efficient Entrapment of Drugs in Dehydration-Rehydration Liposomes 0DRVs), *Int. J. Pharm.* 65, 235–242 (1990).

5) E. Doegito, H. Fessi, M. Appel, F. Puisieux, J. Bolard, and J. P. Devissaguet, "New Techniques for Preparing Submicronic Emulsions—Application to Amphotericine-B,: *STP Pharma Sciences* 4, 155–162 (1994).

6D. M. Lidgate, R. C. Fu, N. E. Byars, L. C. Foster, and J. S. Fleitman, "Formulation of Vaccine Adjuvant Muramyldipeptides. Part 3. Processing Optimization, Characterization and Bioactivity of an Emulsion Vehicle," Pharm Res. 6, 748–752 (1989).

7) H. Talsma, A. Y. Ozer, L. VanBloois, and D. J. Crommelin, "The Size Reduction of Liposomes with a High Pressure Homogenizer (Microfluidizer): Characterization of Prepared Dispersions and Comparison with Conventional Methods," *Drug Dev. Ind. Pharm.* 15, 197–207 (1989).

8) D. M. Lidgate, T. Tranner, R. M. Shultz, and R. Maskiewicz, "Sterile Filtration of a Parenteral Emulsion," *Pharm. Res.* 9, 860–863 (1990).

9) R. Bodmeier, and H. Chen, "Indomethacin Polymeric Nanosuspensions Prepared by Microfluidization," *J. Contr. Rel.* 12, 223–233 (1990).

10) R. Bodmeier, H. Chen, P. Tyle, and P. Jarosz, "Spontaneous Formation of Drug-Containing Acrylic Nanoparticles," *J. Microencap,* 8, 161–170 (1991).

11) F. Koosha, and R. H. Muller, "Nanoparticle Production by Microfluidization," *Archiv Der Pharmazie* 321, 680 (1988).

However, reports are few on reducing mean particle size (hereinafter sometimes abbreviated as MPS) of water-insoluble materials for use in pharmaceutical/diagnostic imaging compositions.

The present invention is directed to a process incorporating the advantages of microfluidizer process over conventional milling processes along with utilizing formulation and/or process parameters necessary for successful particle size reduction of a pharmaceutical suspension.

The primary forces attributed to microfluidization for producing either emulsions or dispersions, and for reducing the MPS of water-insoluble materials include: shear, involving boundary layers, turbulent flow, acceleration and change in flow direction; impact, involving collision of solid elements and collision of particles in the chamber of microfluidizer; and cavitation, involving an increased change in velocity with a decreased change in pressure and turbulent flow. An additional force can be attributed to conventional milling processes of attrition, i.e., grinding by friction. In reference to conventional milling process it is understood that the process involves the use of gravity, attrition and/or media mills, all containing a grinding media.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a process of preparing stable, dispersible, water-insoluble, drug nanoparticles consisting essentially of a crystalline drug substance having a surface modifier adsorbed on the surface thereof comprising the steps of:

a) dispersing a crystalline drug substance in a liquid dispersion medium containing a surface modifier, and

b) subjecting the liquid dispersion medium to the comminuting action of a microfluidizer asserting shear, impact and cavitation forces onto the crystalline drug substance contained in the liquid dispersion medium for a time necessary to reduce the mean particle size of said crystalline drug substance to less than 400 nm.

The particles can be formulated into pharmaceutical compositions exhibiting remarkably high bioavailability.

This invention also provides a stable dispersion consisting essentially of a liquid dispersion medium and the above-described particles dispersed therein.

In a particularly valuable and important embodiment of the invention, there is provided a pharmaceutical composition comprising the above-described particles and a pharmaceutically acceptable carrier therefor. Such pharmaceutical composition is useful in a method of treating mammals.

It is an advantageous feature that a wide variety of surface modified drug nanoparticles free of unacceptable contamination can be prepared in accordance with this invention.

Still another advantageous feature of this invention is that pharmaceutical compositions containing poorly water soluble drug substances are provided which are suitable for intravenous administration techniques.

DETAILED DESCRIPTION OF THE INVENTION

This invention is based partly on the discovery that drug particles having an extremely small effective avenge particle size can be prepared by milling in a microfluidizer in conjunction with a surface modifier, and that such particles are stable and do not appreciably flocculate or agglomerate due to interparticle attractive forces and can be formulated into pharmaceutical compositions exhibiting unexpectedly high bioavailability. While the invention is described herein primarily in connection with its preferred utility, i.e., with respect to nanoparticulate drug substances for use in pharmaceutical compositions, it is also believed to be useful in other applications such as the formulation of particulate cosmetic compositions and the preparation of particulate dispersions for use in image and magnetic recording elements.

The particles of this invention comprise a drug substance. The drug substance exists as a discrete, crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796 cited above.

The Drug Substances

The invention can be practiced with a wide variety of drug substances. The drug substance preferably is present in an essentially pure form. The drug substance must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the drug substance has a solubility in the liquid dispersion medium, e.g. water, of less than about 10 mg/ml, and preferably of less than about 1 mg/ml. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a drug substance is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

Suitable drug substances can be selected from a variety of known classes of drugs including, for example, analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobactefial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radiopharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. Preferred drug substances include those intended for oral administration and intravenous administration. A description of these classes of drugs and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia,* Twenty-ninth Edition, The Pharmaceutical Press, London, 1989, the disclosure of which is hereby incorporated herein by reference in its entirety. The drug substances are commercially available and/or can be prepared by techniques known in the art.

Representative illustrative species of drug substances useful in the practice of this invention include:

17-α-pregno-2,4-dien-20-yno-[2,3-d]-isoxazol- 17-ol (Danazol);

5α,17α,- 1'-(methylsulfonyl)- 1'H-pregn-20-yno[3,2-c]-pyrazol- 17-ol (Steroid A);

piposulfam;

piposulfan;

camptothecin; and

ethyl-3,5-diacetoamido-2,4-6- triiodobenzoate.

In particularly preferred embodiments of the invention, the drug substance is a steroid such as danazol or Steroid A or an antiviral agent.

Surface Modifiers

The particles of this invention contain a discrete phase of a drug substance as described above having a surface modifier adsorbed on the surface thereof. Useful surface modifiers are believed to include those which physically adhere to the surface of the drug substance but do not chemically bond to the drug.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate,carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hyclroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the *Handbook of Pharmaceutical Excipients,* published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Particularly preferred surface modifiers include polyvinyl pyrrolidone, Pluronic F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, Tetronic 908, which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, Aerosol OT, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol P, which is a sodium lauryl sulfate, available from DuPont, Triton X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 80, which is a polyoxyethylene sorbitan fatty acid ester, available from ICI Specialty Chemicals, Carbowax 3350 and 934, which are polyethylene glycols available from Union Carbide. Surface modifiers which have found to be particularly useful include polyvinylpyrrolidone, Pluronic F-68, and lecithin.

The surface modifier is adsorbed on the surface of the drug substance in an amount sufficient to maintain an effective average particle size of less than about 400 nm. The surface modifier does not chemically react with the drug substance or itself. Furthermore, the individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages.

As used herein, particle size refers to a number average particle size of less than about 400 nm as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments of the invention, the effective average particle size is less than about 250 nm. In some embodiments of the invention, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than .400 nm.

The Microfluidizer

In the practice of the present invention the following microfluidizers were used, all supplied by Microfluidics International Corporation:

Model M110-EH, which is a laboratory scale microfluidizer which utilizes an electric hydraulic pump;

Model M-110Y, which is a laboratory scale microfluidizer equipped with a sanitary pressure transducer connected to a digital data acquisition system;

Model M-140K, which is a high pressure microfluidizer with a pressure limit of 40,000 psi; and

Model M-210, which is a pilot plant microfluidizer with a pressure range from 3,000 to 30,000 psi, and with flow rates between 1.9 to 5.7 L/min. It is capable of handling a sample size of 3.8 L or greater.

As indicated, the primary forces attributed to microfluidization by the microfluidizer for producing either emulsions or dispersions, and for reducing mean particle size of water-insoluble materials are:

shear, involving boundary layers, turbulent flow, acceleration and change in flow direction;

impact, involving collision of the particles processed with solid elements of the microfluidizer, and collision between the particles being processed; and

cavitation, involving an increased change in velocity with a decreased change in pressure, and turbulent flow.

An additional force can be attributed to attrition, i.e., grinding by friction.

The M-110Y laboratory scale microfluidizer consists of an air motor connected to a hydraulic pump which circulates the process fluid. The formulation stream is propelled at high pressures (up to 23,000 psi) through a specially designed interaction chamber which has fixed microchannels that focus the formulation stream and accelerate it to a high velocity. Within the chamber the formulation is subjected to intense shear, impact and cavitation, all of which contribute to particle size reduction. After processing, the formulation stream is passed through a heat exchanger coil and can be collected or recirculated through the machine. The microfluidizer was typically used in a continuous processing mode for up to three hour of total processing time. The heat exchanger and interaction chamber were externally cooled with a refrigerated circulating water bath.

The use of microfluidization in pharmaceutical dosage form development has largely been limited to processing of emulsions or liposomes as previously discussed.

The Process of Making the Nanoparticulates

A general procedure for preparing the particles useful in the practice of this invention follows. The drug selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse drug substance selected be less than about 100 gm, as determined by sieve analysis. If the coarse particle size of the drug substance is greater than about 100 gm then it is preferred that the coarse particles of the drug substance be reduced in size to less than 100 gm using a conventional milling method such as airjet or fragmentation milling.

The coarse drug substance selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the drug substance in the liquid medium can vary from about 0.1–60% w/w, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to 90%; and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix then can be transferred to the microfluidizer and circulated continuously first at low pressures, then at maximum capacity having a fluid pressure of from about 3,000 to 30,000 psi until the desired particle size reduction is achieved. The particles must be reduced in size at a temperature which does not significantly degrade the drug substance. Processing temperatures of less than about 30–40° C. are preferred.

There are two methods to collect a slurry and re-pass it in a microfluidizer. The "discreet pass" method collects every pass through the microfluidizer until all of the slurry has been passed through before re-introducing it again to the microfluidizer. This guarantees that every substance or particle has "seen" the interaction chamber the same amount of times. The second method recirculates the slurry by collecting it in a receiving tank and allowing the entire mixture to randomly mix and pass through the interaction chamber. We have found that recirculating a slurry is just as effective as the "discreet pass" method, however, maintaining slurry homogeneity in the receiving tank is important.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

The relative amount of drug substance and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular drug substance and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square meter surface area of the drug substance. The surface modifier can be present in an amount of 0.1–90%, preferably 20–60% by weight based on the total weight of the dry particle.

The resulting dispersion of this invention is stable and consists of the liquid dispersion medium and the above-described particles. The dispersion of surface modified drug nanoparticles can be spray coated onto sugar spheres or onto a pharmaceutical excipient in a fluid-bed spray coater by techniques well known in the art.

Pharmaceutical compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art. These include non-toxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, and the like. A method of treating a mammal in accordance with this invention comprises the step of administering to the mammal in need of treatment an effective amount of the above-described pharmaceutical composition. The selected dosage level of the drug substance for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore, depends upon the particular drug substance, the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors. As noted, it is a particularly advantageous feature that the pharmaceutical compositions of this invention exhibit unexpectedly high bioavailability as illustrated in the examples which follow. Furthermore, it is contemplated that the drug particles of this invention provide more rapid onset of drug action in oral applications and decreased gastric irritancy.

It is contemplated that the pharmaceutical compositions of this invention will be particularly useful in oral and parenteral, including intravenous, administration applica-

tions. It is expected that poorly water soluble drug substances, which prior to this invention, could not have been administered intravenously, may be administered safely in accordance with this invention. Additionally, drug substances which could not have been administered orally due to poor bioavailability may be effectively administered in accordance with this invention.

While applicants do not wish to be bound by theoretical mechanisms, it is believed that the surface modifier hinders the flocculation and/or agglomeration of the particles by functioning as a mechanical or steric barrier between the particles, minimizing the close, interparticle approach necessary for agglomeration and flocculation. Alternatively, if the surface modifier has ionic groups, stabilization by electrostatic repulsion may result. It was surprising that stable drug particles of such a small effective average particle size and free of unacceptable contamination could be prepared by the method of this invention.

Illustrative examples of drug substance microfluidized in the presence of surface active agents and mean particle size of the microfluidized drug substances are shown in Table I.

TABLE I

Microfluidization of Therapeutics

Compound % w/w	Surfactant % w/w	Mean Particle Size
naproxen (2.5%)	HPMC (0.25%)	309 nm (30 min) 301 nm (60 min)
naproxen (2.5%)	HPMC (1.75%)	335 nm (30 min) 307 nm (60 min)
naproxen (20%)	HPMC (2.0%)	314 nm (180 min)
naproxen (20%)	HPC (1.6%)	223 nm (180 min)
naproxen (40%)	PVP K-29/32 (6%)	271 nm (150 min)
WIN 49596 (20%)	SLS (0.2%)	224 nm (60 min)
WIN 63394(6.8%)	F68 (5%)	284 nm (30 min)
Danazol (12%)	DOSS (0.3%)	318 nm (180 min)

wherein
naproxen = 6 methoxy-α-methyl-2-naphthaleneacetic acid
HPMC = hydroxypropylmethylcellulose
HPC = hydroxypropylcellulose
WIN 49596 = (5-α-, 17-α)-1'-(methylsulfonyl)-1'-H-preg-20-yno[3,2-C] pyrazol-17-ol (generic name zanoterone)
WIN 63394 = Benzoic acid, 6-dichloro-[6-methoxy-4-(1-methylethyl)-3-oxo-1,2-benzisothiazol-2-(3H)-yl]methyl S,S, dioxide
SLS = sodium lauryl sulfate
DOSS = sodium dioctylsulfosuccinate

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed is:

1. A process for preparing particles consisting essentially of 99.9–10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of (). 1–9()% by weight and sufficient to maintain an effective average particle size of less than about 400 nm, said process comprises the steps of:

a) preparing a promix of said crystalline drug substance having a particle size of less than about 100 μm and said surface modifier by mixing them in a liquid dispersion medium being selected form the group consisting of water, aqueous salt solutions, safflower oil, ethanol, t-butanol, hexane and glycol;

b) transferring said premix to a microfluidizer having an interaction chamber capable of producing shear, impact, cavitation and attrition forces;

c) subjecting said promix to said forces at a temperature not exceeding 40° C. and a fluid pressure of from about 3,000 to about 30,000 psi by passing said premix through said interaction chamber to reduce the particle size of said drug substance and to obtain a homogeneous slurry thereof:

d) collecting all the slurry from said interaction chamber into a receiving tank;

c) reintroducing said slurry m said receiving tank into said interaction chamber to further subject said slurry to said forces and thereby to decrease the effective average particle size of said drub substance: and

f) repeating said collection and reintroduction steps until said drug substance is reduced to an effective average particle size of less than about 400 nm.

2. The process of claim **1** wherein said particles have an effective particle size of less than 250 nm.

3. The process of claim **1** wherein said particles have an effective particle size of less than 100 μm.

4. The process of claim **1** wherein said drug substance is selected from the group consisting of: analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, contrast media, corticosteroids, cough suppressants, diuretics, dopaminergics, haemostatics, immufiological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, sympathomimetics, thyroid agents, vasoclilators and xanthines.

5. The process of claim **1** wherein said drug substance is a steroid.

6. The process of claim **1** wherein said said drug substance is selected from the group consisting of: (Danazol), 5α,17α,-1'-(methylsulfonyl)- 1'H-pregn-20-yno-pyrazol17-ol, piposulfam, piposulfan, camptothecin and ethyl-3,5-diacetoamido-2,4-6triiodobenzoate.

7. The process of claim **1** wherein said surface modifier is selected from the group consisting of: gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene caster oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone.

8. The process of claim **1** wherein said surface modifier is selected from the group consisting of: an ethylene oxide-propylene oxide block co-polymer, lecithin, an alkyl aryl polyether sulfonate, gum acacia, sodium dodecylsulfate, and a dioctylester of sodium sulfosuccinic acid.

9. A pharmaceutical composition comprising the particles prepared by the process of claim **1** in Combination with a pharmaceutically acceptable carrier.

10. A method of treating a mammal comprising the step of administering to the mammal an effective amount of the pharmaceutical composition of claim **9**.

11. A process for preparing particles consisting essentially of 99.9–10% by weight of a crystalline drug substance

having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm, said process comprises the steps of:

a) preparing a promix of said crystalline drug substance having a particle size of less than about 100 μm and said surface modifier by mixing them in a liquid dispersion medium being selected form the group consisting of water, aqueous salt solutions, safflower oil, ethanol, t-butanol, hexane and glycol;

b) transferring said premix to a microfluidizer having an interaction chamber capable producing shear, impact, cavitation and attrition forces;

c) subjecting said premix to said forces at a temperature not exceeding 40° C. and a fluid pressure of from about 3,000 to about 30,000 psi by passing said premix through said interaction chamber to reduce the particle size of said drug substance and to obtain a homogeneous slurry thereof;

d) collecting a portion of the slurry from said interaction chamber into a receiving tank:

e) reintroducing said portion of the slurry into said interaction chamber; repeating said collection and reintroduction steps in a continuous process until said drug substance is reduced to an effective average particle size of less than about 400 nm.

12. The process of claim **11** wherein said particles have an effective particle size of less than 250 nm.

13. The process of claim **11** wherein said particles have an effective particle size of less than 100 μm.

14. The process of claim **11** wherein said drug substance is selected from the group consisting of: analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, antic oagul ants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, contrast media, corticosteroids, cough suppressants, diuretics, dopaminergics, haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, sympathomimetics, thyroid agents, vasodilators and xanthines.

15. The process of claim **11** wherein said drug substance is a steroid.

16. The process of claim **11** wherein said drug substance is selected from the group consisting of: (Danazol), 5α, 17α,- 1'-(methylsulfonyl)- 1'H-pregn-20-yno-pyrazol-17-ol, piposulfam, piposulfan, camptothecin and ethyl-3,5-diacetoamido-2,4-6-triiodobenzoate.

17. The process of claim **11** wherein said surface modifier is selected from the group consisting of: gelatin, casein, locithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene caster oil derivatives, polyoxyethylene sorbitan htty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidel silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone.

18. The process of claim **11** wherein said surface modifier is selected from the group consisting of: an ethylene oxide-propylene oxide block co-polymer, lecithin, an alkyl aryl polyether sulfonate, gum acacia, sodium dodecylsulfate, and a dioctylester of sodium sulfosuccinic acid.

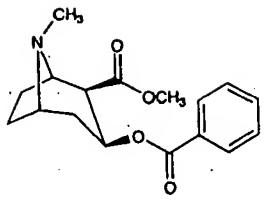
19. A pharmaceutical composition comprising the particles prepared by the process of claim **11** in combination with a pharmaceutically acceptable carrier.

20. A method of treating a mammal comprising the step of administering to the mammal an effective amount of the pharmaceutical composition of claim **19**.

* * * * *

6. The Merck Index 12th ed., Merck & Co. pp. 416-417 (1996).

The Merck Index 12th 1996



Monoclinic tablets from alcohol, mp 98°. Volatile, esp above 90°, but the sublimate is not crystalline. $bp_{0.1}$ 187-188°. $[\alpha]_D^{18}$ −35° (50% alcohol); $[\alpha]_D^{20}$ −16° (c = 4 in chloroform). Aq solns are alkaline to litmus. pKa (15°) 8.61. pKb (15°) 5.59. One gram dissolves in 600 ml water, 270 ml water at 80°, 6.5 ml alcohol, 0.7 ml chloroform, 3.5 ml ether, 12 ml oil turpentine, 12 ml olive oil, 30-50 ml liquid petrolatum. Also sol in acetone, ethyl acetate, carbon disulfide. LD_{50} i.v. in rats: 17.5 mg/kg (Rose).

Hydrochloride, $C_{17}H_{21}NO_4.HCl$, *cocaine muriate.* Crystals, granules, or powder; saline, slightly bitter taste; numbs tongue and lips. mp ~195°. $[\alpha]_D$ −72° (c = 2 in aq soln pH 4.5). One gram dissolves in 0.4 ml water; 3.2 ml cold, 2 ml hot alcohol; 12.5 ml chloroform. Also sol in glycerol, acetone. Insol in ether or oils. Avoid heat in preparing soln as it decomposes. Preserve in well-closed, light-resistant containers.

Nitrate dihydrate, $C_{17}H_{22}N_2O_7.2H_2O$, crystals, mp 58-63°. Freely sol in water or alcohol; slightly sol in ether.

Sulfate, $C_{17}H_{21}NO_4.H_2SO_4$, white, granular, crystalline powder. Sol in water or alcohol.

Caution: May be habit forming. Cocaine and its derivatives are controlled substances listed in the U.S. Code of Federal Regulations, Title 21 Parts 329.1 and 1308.12 (1995).

THERAP CAT: Anesthetic (local).

THERAP CAT (VET): Topical anesthetic (ophthalmic).

2518. Cocculus. Fish-berry; Indian berry; *Cocculus indicus;* oriental berry. Dried fruit of *Anamirta cocculus* (L.) Wight & Arn., *Menispermaceae. Habit.* East Indies, Malay Archipelago. *Constit.* Menispermine, paramenispermine, about 1% picrotoxin, picrotoxic acid, cocculine alkaloid, about 50% fat. *Poisonous!*

THERAP CAT: CNS and respiratory stimulant.

2519. Cochineal. The dried female insect, *Coccus cacti* L., enclosing the young larvae. *Habit.* Mexico, Central America; cultivated in West Indies, Canary Islands, Algiers, and Southern Spain. About 70,000 insects to 1 lb. *Constit.* About 10% carminic acid, about 2% coccerin (a wax), about 10% fat. The coloring matter—alkali carminate—is contained only in the fatty parts of the insect and in the yolk of the eggs, to the extent of 10-14%.

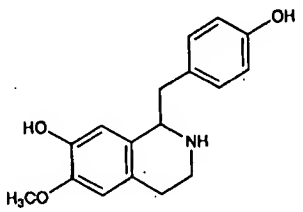
USE: Coloring food products and toilet preparations; the source of carmine and carminic acid for manuf red and pink inks and lakes.

2520. Cocillana. Dried bark of *Guarea rusbyi* (Britt.) Rusby, *Meliaceae. Habit.* Bolivia. *Constit.* Rusbyine, about 2.5% resins, about 2.5% fat, tannin.

THERAP CAT: Expectorant.

THERAP CAT (VET): Has been used as an expectorant.

2521. Coclaurine. *(S)-1,2,3,4-Tetrahydro-1-[(4-hydroxyphenyl)methyl]-6-methoxy-7-isoquinolinol;* 1-(*p*-hydroxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline; machiline. $C_{17}H_{19}NO_3$; mol wt 285.34. C 71.56%, H 6.71%, N 4.91%, O 16.82%. Isolated as the racemate from species of *Machilus (Lauraceae)* and *Cocculus (Menispermaceae).* First isoln from *C. laurifolius* D.C. believed to be of the *d*-form: Kondo, Kondo, *J. Pharm. Soc. Japan* no. 524, 876 (1925), *C.A.* **20**, 604[7] (1926); *see also* Johns *et al., Aust. J. Chem.* **20**, 1729 (1967). Structure: Kondo, Kondo, *J. Pharm. Soc. Japan* **48**, 1156 (1928); Tomita, Kusuda, *ibid.* **72**, 280 (1952). Synthesis: Kratzl, Billek, *Monatsh.* **82**, 568 (1951); Finkelstein, *J. Am. Chem. Soc.* **73**, 550 (1951). Identity with machiline: Tomita *et al., J. Pharm. Soc. Japan* **83**, 218 (1963), *C.A.* **59**, 2874a (1963). Crystal structure and absolute configuration: Fridrichsons, Mathieson, *Tetrahedron* **24**, 5785 (1968).



Plates, tablets from alc, mp 220-221°. Sol in hot alc, hot acetone; slightly sol in water, alc, chloroform, ether, acetone; practically insol in benzene, petr ether.

Hydrochloride, $C_{17}H_{19}NO_3.HCl$, crystals, mp 263-264°.

2522. Cocoa. A powder prepd from the roasted and cured kernels of ripe seeds of *Theobroma cacao* L. and other species of *Theobroma, Sterculiaceae.* For bibliography *see* Cacao Shell.

Brownish powder of chocolate odor and taste.

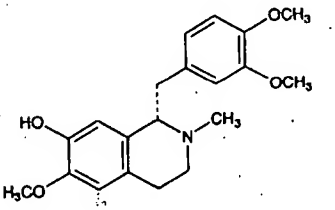
USE: In nutrient beverages; as flavoring.

2523. Coconut Oil. Copra oil. Expressed oil from kernels of *Cocos nucifera* L., *Palmae. Constit.* Trimyristin, trilaurin, tripalmitin, tristearin; also various other glycerides.

White, semisolid, lard-like fat; stable to air. Remains bland and edible for several years under ordinary storage conditions. d_4^0 0.903. mp 21-25°. n_D^{40} 1.4485-1.4495. Sapon. no. 255-258. Iodine no. 8-9.5. Acid no. not over 6. Surface tension (20°): 33.4 dyn/cm; (80°): 28.4 dyn/cm. Practically insol in water, 95% alc, more sol in abs alc; very sol in chloroform, ether, carbon disulfide. Soly data: Rao, Arnold, *J. Am. Oil Chem. Soc.* **33**, 389 (1956).

USE: Manuf soap, edible fats, chocolate, candies; in baking instead of lard; in candles and night lights; in dyeing cotton; as an ointment base; in hair dressing; in massage.

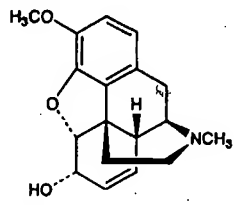
2524. Codamine. *(S)-1-[(3,4-Dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6-methoxy-2-methyl-7-isoquinolinol; 1,2,3,-4-tetrahydro-6-methoxy-2-methyl-1-veratryl-7-isoquinolinol.* $C_{20}H_{25}NO_4$; mol wt 343.42. C 69.95%, H 7.34%, N 4.08%, O 18.64%. Minor opium alkaloid. Constitutes about 0.003% of Turkish opium. Isoln: Hesse, *Ann.* **282**, 213 (1894). Structure: Späth, Epstein, *Ber.* **59B**, 2791 (1926). Synthesis: Schöpf, Thierfelder, *Ann.* **537**, 143 (1939); Billek, *Monatsh.* **87**, 106 (1956).



dl-Form, large, six-sided prisms from ether, mp 127°. Very sol in alcohol, chloroform. Somewhat sol in boiling water. In soln codamine reacts strongly basic. The salts are bitter in taste, whereas the base is said to be tasteless.

2525. Codeine. *(5α,6α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol;* methylmorphine; morphine monomethyl ether; morphine 3-methyl ether; Codicept. $C_{18}H_{21}NO_3$; mol wt 299.37. C 72.22%, H 7.07%, N 4.68%, O 16.03%. Present in opium from 0.7 to 2.5%, depending on the source, but mostly prepd by methylation of morphine, *q.v.* Discussion of structure and bibliography: Small, Lutz, "Chemistry of the Opium Alkaloids," *U.S. Public Health Reports,* Suppl. No. 103, Washington (1932). Prepn of (+)-codeine and racemate: Goto, Yamamoto, *Proc. Japan Acad.* **30**, 769 (1954), *C.A.* **50**, 1052h (1956); of (−)-form: E. J. Bijsterveld, H. J. Sinnige, *Rec. Trav. Chim.* **95**, 24 (1976); H. C. Beyerman *et al., ibid.* **97**, 127 (1978). Manuf from morphine: W. R. Heumann, *Bulletin on Narcotics* X, 15 (1958). Facile synthesis from thebaine, *q.v.:* W.

G. Dauben *et al., J. Org. Chem.* **44**, 1567 (1979). Toxicity of the hydrochloride: Eddy, Sumwalt, *J. Pharmacol. Exp. Ther.* **67**, 127 (1939). Comprehensive description of codeine and codeine phosphate, *q.v.*: F. J. Muhtadi, M. M. A. Hassan, *Anal. Profiles Drug Subs.* **10**, 93-138 (1981).



Monohydrate, orthorhombic sphenoidal rods or tablets (octahedra) from water or dil alcohol, mp 154-156° (after drying at 80°). Sublimes (when anhydr) at 140-145° under 1.5 mm pressure. Melts to oily drops when heated in an amount of water insufficient for complete soln, crystallizes on cooling. d_4^{20} 1.32. $[\alpha]_D^{15}$ −136° (c = 2 in alcohol), $[\alpha]_D^{15}$ −112° (c = 2 in chloroform). pK (15°) 6.05; pH of satd aq soln 9.8. One gram dissolves in 120 ml water, 60 ml water at 80°, 2 ml alcohol, 1.2 ml hot alcohol, 13 ml benzene, 18 ml ether, 0.5 ml chloroform; freely sol in amyl alcohol, methanol, dil acids. Almost insol in petr ether or in solns of alkali hydroxides.

Acetate, $C_{20}H_{25}NO_5$. Dihydrate, crystals; acetic acid odor. Sol in water, alc. Loses acetic acid on keeping, then becomes incompletely sol in water. *Keep tightly closed.*

Hydrobromide, $C_{18}H_{21}NO_3.HBr$. Dihydrate, crystals. Anhydr, mp 190-192°. $[\alpha]_D^{22}$ −96.6°. One gram dissolves in 60 ml water, 110 ml alcohol. pH about 5.

Hydrochloride, $C_{18}H_{21}NO_3.HCl$. Dihydrate, small needles, mp ~280° with some decompn. $[\alpha]_D^{22}$ −108°. One gram dissolves in 20 ml water, 1 ml boiling water, 180 ml alcohol. pH about 5. LD_{50} s.c. in mice: 300 mg/kg (Eddy, Sumwalt).

Salicylate, $C_{25}H_{27}NO_6$, white, crystalline powder. Slightly sol in water; freely sol in alcohol or ether.

Caution: May be habit forming. This is a controlled substance (opiate) listed in the U.S. Code of Federal Regulations, Title 21 Parts 329.1 and 1308.12 (1995).

THERAP CAT: Analgesic (narcotic); antitussive.

THERAP CAT (VET): Analgesic (narcotic); antitussive.

2526. Codeine Methyl Bromide. Eucodin. $C_{19}H_{24}BrNO_3$; mol wt 394.31. C 57.88%, H 6.13%, Br 20.26%, N 3.55%, O 12.17%. $C_{18}H_{21}NO_3.CH_3Br$.

Crystals, mp ~260°. Sol in 2-3 parts water, in hot methanol, sparingly in alc. Insol in chloroform, ether.

Caution: May be habit forming. This is a controlled substance (opium derivative) listed in the U.S. Code of Federal Regulations, Title 21 Parts 329.1, 1308.11 (1995).

THERAP CAT: Analgesic (narcotic); antitussive.

2527. Codeine *N*-Oxide. Genocodeine; genkodein; Codeigene. $C_{18}H_{21}NO_4$; mol wt 315.37. C 68.55%, H 6.71%, N 4.44%, O 20.29%. Prepn from codeine and 30% hydrogen peroxide: Freund, Speyer, *Ber.* **43**, 3313 (1910); Kelentey *et al.; Arzneimittel-Forsch.* **7**, 594 (1957).

Platelets from water, mp 231-232°.

Monohydrate, crystals from alc, mp 215°. $[\alpha]_D^{18}$ −97.1° (c = 2.1 in water).

Hydrochloride monohydrate, $C_{18}H_{21}NO_4.HCl.H_2O$, crystals, loses crystal water at 110°, mp 219-220°. $[\alpha]_D^{20}$ −105.8° (c = 2 in water). One gram dissolves in 9.5 ml water.

Note: This is a controlled substance (opium derivative) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.11 (1995).

THERAP CAT: Antitussive.

2528. Codeine Phosphate. Galcodine. $C_{18}H_{24}NO_7P$; mol wt 397.36. C 54.41%, H 6.09%, N 3.52%, O 28.18%, P

Sesquihydrate, very efflorescent, small crystals or cryst powder. One gram dissolves in 2.3 ml water, 0.5 ml water at 80°, 325 ml alcohol, 125 ml boiling alcohol, 4500 ml chloroform, 1875 ml ether. pH of a 2% aq soln: 4.6. *Keep well closed.*

Note: This is a controlled substance (opiate) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.12 (1995).

THERAP CAT: Analgesic (narcotic); antitussive.

THERAP CAT (VET): Analgesic (narcotic); antitussive.

2529. Codeine Sulfate. $C_{36}H_{44}N_2O_{10}S$; mol wt 696.82. C 62.05%, H 6.36%, N 4.02%, O 22.96%, S 4.60%.

Trihydrate, crystals or cryst powder. One gram dissolves in 30 ml water, 6.5 ml water at 80°, 1300 ml alc. Insol in chloroform or ether. pH: 5.0. Store in airtight containers; protect from light.

Note: This is a controlled substance (opiate) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.12 (1995).

THERAP CAT: Analgesic (narcotic); antitussive.

THERAP CAT (VET): Analgesic (narcotic); antitussive

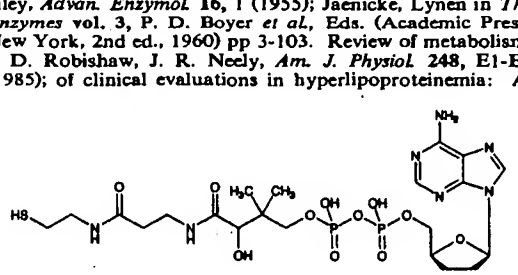
2530. Cod Liver Oil. Gaduol; Tunol. The partially destearinated fixed oil expressed from fresh livers of *Gadus morrhua* L., and other species of *Gadidae. Constit.* Most important are vitamins A and D, each gram containing at least 850 U.S.P. units vitamin A (255 μg) and at least 85 U.S.P. units vitamin D (2.125 μg); glycerides of palmitic, stearic, etc. acids (≃19% saturated fatty acids; remainder unsaturated); cholesterol, batyl alcohol esters. Source of omega 3 fatty acid: N. Haagsma *et al., J. Am. Oil Chem. Soc.* **59**, 117 (1982). Brief description: D. Hilditch, P. Williams, *The Chemical Constitution of Natural Fats* (Wiley-Interscience, New York, 4th ed., 1964) p 43; *Bailey's Industrial Oils & Fat Products* Vol. 1, D. Swern (Wiley-Interscience, New York, 4th ed., 1979) pp 451-453.

Pale-yellow, thin liq; bland, slightly fishy taste and odor. Becomes yellow, acquires a somewhat disagreeable odor on exposure to air and light. d 0.918-0.927. n_D^{40} 1.4705-1.4745. Sapon no. 180-190. Iodine no. 145-180. Acid no. not over 1.2. Slightly sol in alcohol; freely sol in chloroform, ether, carbon disulfide, ethyl acetate, petr ether.

THERAP CAT: Vitamins A and D source.

THERAP CAT (VET): Source of vitamins A and D. Locally to promote healing.

2531. Coenzyme A. CoA. $C_{21}H_{36}N_7O_{16}P_3S$; mol wt 767.54. C 32.86%, H 4.73%, N 12.77%, O 33.35%, P 12.11%, S 4.18%. An essential cofactor in enzymatic acetyl transfer reactions. Synthesized in cells from pantothenate, ATP and cysteine. Found ubiquitously in mammalian cells. Isoln from animal sources: Lipmann *et al., J. Biol. Chem.* **167**, 869 (1947); **186**, 235 (1950). Many microorganisms contain large amounts of the coenzyme. Isoln from *Streptomyces fradiae:* Kaplan, Lipmann, *ibid.* **174**, 37 (1948). Purifications: De Vries *et al., J. Am. Chem. Soc.* **72**, 4838 (1950); Gregory *et al., ibid.* **74**, 854 (1952). Structure: Baddiley *et al., Nature* **171**, 76 (1953). Total synthesis: Moffatt, Khorana, *J. Am. Chem. Soc.* **81**, 1265 (1959); **83**, 663 (1961); Shimizu *et al., Chem. Pharm. Bull.* **13**, 1142 (1965). Reviews: Lipmann, *Bacteriol. Rev.* **17**, 1-16 (1953); Baddiley, *Advan. Enzymol.* **16**, 1 (1955); Jaenicke, Lynen in *The Enzymes* vol. 3, P. D. Boyer *et al.*, Eds. (Academic Press, New York, 2nd ed., 1960) pp 3-103. Review of metabolism: J. D. Robishaw, J. R. Neely, *Am. J. Physiol.* **248**, E1-E9 (1985); of clinical evaluations in hyperlipoproteinemia: A.



7. U.S. Patent No. 5,776,563 to Buhl et al.

US005776563A

United States Patent [19]

Buhl et al.

[11] **Patent Number: 5,776,563**

[45] **Date of Patent: Jul. 7, 1998**

[54] **DRIED CHEMICAL COMPOSITIONS**

[75] Inventors: **Steven N. Buhl**, Cupertino; **Bhaskar Bhayani**, Fremont; **Chi-Sou Yu**, Saratoga; **Thuy N. Tang**, San Jose, all of Calif.

[73] Assignee: **Abaxis, Inc.**, Sunnyvale, Calif.

[21] Appl. No.: **466,155**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 134,574, Oct. 8, 1993, abandoned, which is a continuation-in-part of Ser. No. 747,179, Aug. 19, 1991, Pat. No. 5,413,732.

[51] **Int. Cl.**[6] **B65D 79/00**

[52] **U.S. Cl.** **428/34.1**; 206/527

[58] **Field of Search** 206/527; 428/34.1

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3,721,725	3/1973	Briggs	264/6
3,819,488	6/1974	Rush	195/103.5
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4,115,537	9/1978	Driscoll	424/1
4,295,280	10/1981	Krupey	34/5
4,351,158	9/1982	Hurwitz	62/60
4,588,696	5/1986	Eskelson	436/130
4,678,812	7/1987	Bollin, Jr.	514/777
4,712,310	12/1987	Roy	34/5
4,716,119	12/1987	Rehner	436/16
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4,762,857	8/1988	Bollin, Jr	514/777
4,820,627	4/1989	McGeehan	435/4
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Primary Examiner—D. S. Nakarani

Attorney, Agent, or Firm—Townsend and Townsend and Crew

[57] **ABSTRACT**

The present invention provides dried chemical compositions comprising dried beads. Typically, the beads comprise reagents suitable for analysis of biological samples, in particular analysis of blood samples in centrifugal analyzers.

4 Claims, No Drawings

DRIED CHEMICAL COMPOSITIONS

The present application is a continuation of application U.S. Ser. No. 08/134,574 filed Oct. 8, 1993, abandoned, which is a continuation in part of application U.S. Ser. No. 07/747,179; filed Aug. 19 1991, now U.S. Pat. No. 5,413,732, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to novel compositions comprising dried chemical compounds and to methods for their preparation. In particular, it relates to novel dried beads useful in a number of applications such as preparation of pharmaceutical compositions or analytical reagents.

In preparing chemical compounds for various uses such as convenient and efficient testing of clinical biological samples, it is frequently important to obtain dry chemical blends in uniform, discrete amounts. These compositions must be efficiently and economically prepared in small precisely measured quantities. Chemical compositions comprising organic materials, however, tend to spoil or degrade on storage, thus creating quality control problems. Thus, various chemical compositions are typically provided in dried form to increase stability. Current technology for producing dry chemical blends involves procedures such as dry blending, spray drying, or fluid bed drying. All three of these procedures, however, have limitations that make them costly, inefficient or difficult to carry out.

In dry blending technology, it is difficult to obtain homogeneous blends of chemicals that have different densities. Moreover, homogeneity is particularly difficult to achieve when very small amounts of ingredients are mixed with large amounts of others. Once made homogeneous, it is extremely difficult to reproducibly (within 1 percent) dispense small amounts (less than about 10 mg) of the blended chemicals.

Spray drying technology provides more homogenous blends of chemicals because the reagents are first dissolved in liquid. Using spray drying, however, it is difficult and costly to obtain precisely sized amounts of blended chemicals. As generally practiced, this process yields particles with size distributions having coefficients of variation greater than 20 percent. The resulting particles have to be reprocessed (usually agglomerated) to obtain uniform particle sizes. After agglomeration, the particles are generally less soluble than the original spray dried particles. Moreover, these procedures typically use fluorocarbon cryogenic solutions which are hazardous to the environment.

Fluid bed technology relies upon spraying a liquid reagent blend onto a particle and drying the liquid to obtain a particle coated with the blended reagents. Using this procedure, it is difficult to obtain uniformly sized particles and to produce a uniform coating.

Of particular interest to the present invention are reagents useful in analyzing biological samples, such as blood plasma or serum, in centrifugal analyzers. The rotors used in such analyzers measure volumes of the sample to be tested, mix the sample with an appropriate diluent and separate fluid from cellular components. The rotors also provide a plurality of separate test wells containing chemical reagents in which discrete volumes are optically tested.

Analysis of biological samples in the test wells of centrifugal rotors impose a number of requirements on the reagents used for analysis. In particular, because the analysis

is typically highly automated, speed of analysis is at a premium. In addition, many clinical diagnostic analyses require that measurements be made within a short time after the sample is added to the reagent. Thus, the dried reagent preparations must dissolve quickly in the sample solution. In addition, rapid rehydration of the reagents can cause bubble formation, which adversely affects results by interfering with optical measurement.

The prior art thus lacks dried chemical compositions which avoid the above problems. In addition, the prior art lacks economical and reliable dried chemical which dissolve quickly in sample solutions. The present application addresses these and related problems.

2. Description of Background Art

U.S. Pat. Nos. 3,721,725 and 3,932,943 relate to methods for producing lyophilized reagents comprising spraying a solution containing the reagents into a moving bath of fluorocarbon refrigerants and lyophilizing the resultant frozen droplets. U.S. Pat. No. 4,848,094 discloses methods for the generation of essentially spherical frozen droplets and improved methods for removing frozen droplets from a cryogenic liquid. U.S. Pat. No. 4,655,047 describes methods for freezing drops of relatively thick liquids by dropping them from a small height into a cryogenic material. U.S. Pat. No. 3,819,488 provides stable lyophilized diagnostic compositions for determining glutamic oxalic transaminase and glutamic pyruvic transaminase activities. U.S. Pat. No. 4,588,696 relates to preparation of tablets used in testing for formaldehyde and/or glutaraldehyde. U.S. Pat. Nos. 4,295,280, 4,351,158, and 4,712,310 all relate to methods for preparing homogenous preparations comprising compounds which are incompatible. U.S. Pat. No. 4,820,627 discloses a fluidized bed process for preparing particles suitable for tableting into diagnostic reagents. U.S. Pat. No. 4,115,537 relates to diagnostic tablets containing ion exchange resins. U.S. Pat. No. 4,755,461 is directed to tableted blood plasma compositions. U.S. Pat. Nos. 4,678,812 and 4,762,857 both relate to diagnostic tablets comprising trehalose as an excipient and stabilizer. The use of TRITONO® X-100 is also disclosed. U.S. Pat. No. 4,716,119 discloses the addition of tetramethylammonium acetate to blood serum. Romanian Patent Appln. No. 85,155 relates to enzymatic alkaline phosphatase reagent tablets comprising p-nitrophenyl phosphate. Driscoll et al., *Clin. Chem.*, 29:1609–1615 (1983) discloses an instrument/reagent system comprising tableted reagents for performing photometric assays.

SUMMARY OF THE INVENTION

The present invention provides methods for forming dried chemical compositions. The method comprise forming a solution comprising a desired compound, dispensing uniform, precisely measured drops of the solution into a cryogenic liquid, preferably unagitated liquid nitrogen, and drying the frozen drops to form dried beads comprising the compound.

The step of drying is preferably accomplished by lyophilizing the frozen drops for about 4 hours to about 24 hours at about 50 to about 450 mTorr.

A variety of solutions and compounds can be used in the methods. Typically, the solution is an aqueous solution and the compound is a reagent for the analysis of a biological sample. Exemplary compounds include sodium fluoride and potassium oxalate.

The dried beads produced by the methods typically have a mean diameter between about 1.5 mm and about 5 mm and are uniform in size and weight. The coefficient of weight

variation of the beads is preferably less than about 3.0%. The uniform, precisely measured drops used to form the beads typically have a volume between about 1.5 μl and about 25 μl. To increase uniformity, the aqueous solution can be degassed before dispensing the drops.

The beads typically comprise fillers in a concentration sufficient to facilitate formation of a chemical lattice in the beads. Preferred fillers include polyethylene glycol, myo-inositol, polyvinylpyrrolidone, dextran, sodium cholate, mannitol, bovine serum albumin, trehalose, or a combination thereof.

The beads may also comprise a surfactants at a concentration sufficient to inhibit bubble formation when the dried beads dissolve. Exemplary surfactants include octoxynol 9 or polyoxyethlene 9 lauryl ether.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides dried chemical composition, typically in the form of beads. The compositions can be used in any application in which stable, dried chemical compositions are required. Because the compositions are dry and free-flowing, they may be used to provide precisely measured quantities of particular compositions. In addition, the compositions are typically in the form of a dried homogenous, mixture of components in a precise ratio. Thus, the problems of accurately dispensing dry mixtures is avoided using the compositions of the invention.

The compositions can comprise essentially any compound which can be prepared in a liquid solution, dispensed as drops into a cryogenic liquid and dried. For instance, the compositions may comprise therapeutically active compounds for use in pharmaceutical compositions. Various biological materials can also be included in the compositions. In addition, the compositions can comprise reagents for analysis of various samples such as biological, chemical, soil samples and the like. In one embodiment they are used in centrifugal rotors and analyzers which allow rapid and economical analysis of blood samples.

In some embodiments, the dried compositions are provided in the form of beads or spheres that comprise a chemical lattice to facilitate rapid and complete dissolution of the spheres in an aqueous solution. They also comprise a surfactant at a concentration sufficient to inhibit bubble formation as the spheres dissolve. The beads may be used in combination with diluent solutions comprising isotonic concentrations of compounds having substantially no effect on the assays.

The dried compositions of the invention are particularly useful in the preparation of pharmaceutical compositions comprising any of a number of therapeutically active compounds, such as analgesics, steroidal and non-steroidal anti-inflammatory compounds, immunosuppressants, chemotherapeutic agents, antibiotics, vitamins and other nutritional additives (e.g., trace elements and amino acids), plasma fractions (e.g., Factor IX) and hormones.

Methods and compounds for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in for example, *Remington's Pharmaceutical Science*, 17th ed., Mack Publishing Company, Easton, Pa. (1985), which is incorporated herein by reference.

The compositions of the invention are prepared as formulations comprising pharmaceutically acceptable media, for example, saline, phosphate buffered saline (PBS), Ringer's solution, dextrose/saline, Hank's solution, and glucose. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents, detergents, and the like. Additives may also include additional active ingredients, e.g., bactericidal agents, or stabilizers.

The pharmaceutical compositions can be prepared for transdermal or parenteral administration, e.g., intravenously, subcutaneously, or intramuscularly. Orally administratable forms may also be desired and can be provided by modifying the composition to bypass the stomach environment. The composition can be used for prophylactic and/or therapeutic treatment. Typically, the compositions comprise the therapeutically active compound dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The concentration of the therapeutically active compound will vary widely, depending upon the compound being administered, the form of administration, the condition being treated and the like. The concentration will also depend upon desired fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, glycol and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10–95% of active ingredient.

The compositions of the invention are also useful in preservation of biological materials in dried form. For instance, the dried beads of the invention may comprise biological samples such as blood or plasma, urine, tissue samples, various proteins (e.g., blood factors, immunoglobulins, enzymes and the like) spinal fluid, sputum, genetic material (DNA or RNA) or other samples suitable for analysis or other uses.

The compositions are also suitable for providing precisely measured aliquots of compounds to be mixed with a sample to preserve the sample or otherwise keep it in a condition suitable for later analysis. For instance, the beads of the invention may comprise anticoagulants (e.g., potassium oxalate, lithium heparin) or metabolic inhibitors (e.g., sodium fluoride) for use in blood collection tubes.

The dried beads of the invention can also comprise reagents used in the analysis of a wide variety of compositions. Examples include the analysis of soil samples, industrial chemicals, food chemicals, agricultural chemicals and the like. In one embodiment, the beads and diluents of the present invention are used in centrifugal analyzers for optically analyzing biological fluids, in particular blood plasma or serum. Centrifugal rotors used in such analyzers typically comprise means for mixing the blood with an appropriate diluent and separating plasma from cellular material. The rotors also provide for distribution of the diluted plasma into a plurality of cuvettes within the rotor so that different optical analytic procedures may be performed without having to transfer aliquots of the fluid from the apparatus. One or more reagent beads comprising the reagents necessary for a desired assay are provided in each cuvette.

The rotors and methods described in the following U.S. Patents are preferably used: U.S. Pat. Nos. 5,061,381, 5,173,193, 5,186,844, and 5,122,284. The entire disclosure of these patents are incorporated herein by reference. The above applications disclose centrifugal rotors for separating plasma from whole blood that include a plurality of internal chambers and passages for combining blood plasma or serum with one or more reagents and distributing the plasma or serum to a plurality of individual test wells. The chambers and passages necessary for separating the whole blood into plasma are located radially outward from metering chambers that deliver precisely measured volumes of blood and/or diluent to a separation chamber. The separation chamber includes a radially-outward cell trap. Spinning of the rotor causes the cellular components of the whole blood to be sequestered in the cell trap. The separated plasma is then delivered to a plurality of test wells or cuvettes. The above separation and aliquoting steps typically occur as a result of centrifugal force generated by the spinning rotor.

The compositions of the present invention in combination with the rotors described above are particularly suitable for analyzing blood plasma or diluted blood plasma. They are also useful with a wide variety of other biological fluids, such as urine, sputum, semen, saliva, ocular lens fluid, cerebral fluid, spinal fluid, amniotic fluid, and tissue culture media, as well as food and industrial chemicals, and the like.

The compositions of the present invention are particularly suitable for performing a wide variety of analytic procedures which are beneficially or necessarily performed on chemical, soil or biological samples, such as blood plasma or diluted plasma. The analytic procedures will generally require that the sample be combined with one or more reagents so that some detectable change occurs in the sample which may be related to measurement of a particular component or characteristic of the sample. For instance, the sample may undergo a optically detectable reaction or other change which results in a change in color, fluorescence, luminescence, or the like, which may be measured by conventional spectrophotometers, fluorometers, light detectors, etc.

In some cases, immunoassays and other specific binding assays may be performed. In these embodiments, the compositions of the invention will comprise antibodies, or fragments thereof, which are used to detect the presence of an antigen in the sample.

Generally, the procedures must be homogeneous and do not require a separation step. In other cases, it will be possible to accommodate heterogeneous assay systems by providing a means to separate blood plasma, a precipitate, or bound material from the test wells after an immunological reaction step has occurred.

If blood is used as the biological sample, conventional blood assays can be performed such as glucose, lactate dehydrogenase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), blood urea (nitrogen) (BUN), total protein, alkalinity, alkaline phosphatase, c-reactive protein, bilirubin, calcium, chloride, sodium, potassium, magnesium, and the like. This list is not exhaustive and is intended merely as being exemplary of the assays which may be performed using the apparatus and method of the present invention. Usually, these tests will require that the blood plasma be combined with one or more reagents which result in a visually detectable, usually photometrically detectable, change in the plasma.

To prepare the compositions of the invention, a solution, typically an aqueous solution, comprising the chemicals to be dried is prepared. To ensure uniform composition of the dried beads, the solution must be homogeneous and all constituents must be fully dissolved or in suspension. Individual drops of the solution are then dispensed into a cryogenic liquid, preferably liquid nitrogen. A cryogenic liquid as used herein refers to a liquified gas having a normal boiling point below about −75° C., preferably below about −150° C.

The frozen masses are then dried, typically lyophilized, to produce the dried beads. The beads typically comprise less than about 6% residual moisture, preferably less than about 3%. Lyophilization or drying step is not a critical aspect of the invention and can be carried out according to any of a number of standard procedures known in the art. Typically, the frozen drops are lyophilized for about 4 hours to about 24 hours at about 50 to about 450 mTorr, preferably, about 6 hours at about 200 mTorr.

The drops are uniform and precisely measured so that the resulting dried beads have uniform mass. When the drops are uniform and precisely measured, the imprecision of the mass (coefficient of weight variation) of the beads prepared from the drops is less than about 3%, and preferably between about 0.3% and about 2.5%. To further decrease the coefficient of weight variation, the aqueous solution is preferably degassed using a vacuum pump or vacuum line before the drops of solution are dispensed.

To obtain values for coefficient of weight variation, known quantities of beads are weighed. The coefficient of variation (C.V.) is then determined as follows:

$$C.V. = J\sqrt{x} \times 100$$

wherein

$$J = \text{standard deviation (for } n \text{ bead)} = \left[\frac{(x-\bar{x})^2}{n-1} \right]^{1/2}$$

x=weight of one bead

$\bar{x}$=mean (for "n" bead)=$\Sigma x/n$

The uniformity of the beads produced by this method obviates the need for an additional tableting step to obtain uniform size. The drops can be dispensed by any of a number of means which provide the necessary precision. Typically, an IVEK model AAA pump (N. Springfield, Vt.) is used. The solution is usually dispensed in discrete drops having a volume between about 1.5 μl and about 25 μl, preferably between about 2.5 μl and about 10 μgl. The exact volume of the drops will depend upon the particular application. For instance, in preparing reagent beads for total protein determinations, 2.96 μl drops are typically used, for C-reactive protein and alkaline phosphatase determinations, 2.67 μl are used. Volumes appropriate for other tests are as follows: SGOT, 4.0 μl; potassium, 4.0 μl; creatinine, 4.0 ml; bilirubin, 2.667 μl; amylase, 2.667 μl; cholesterol, 2.667 μl; uric acid, 3.478 μl; and glucose, 2.065 μl.

As noted above, the methods of the invention can be used to make chemical beads for aliquoting a variety of chemicals. For example, volumes appropriate for food and agricultural chemicals include lactic acid, 4.0 μl and citric acid 2.065 μl. In the case of the anticoagulant and metabolic inhibitors for use in blood collection tubes, the volume of the anticoagulant potassium oxalate should be 20 μl and the volume of the metabolic inhibitor sodium fluoride is 12 μl.

The beads of the invention can be made in a wide range of sizes, depending upon the volume of solution used to

prepare them. Typically, the beads will have a diameter of less than about 10 mm, usually less than about 5, or in some applications about 3.5 mm. The minimum size is typically about 1.5 mm.

The beads dissolve quickly in a sample solution. In the analysis of a biological sample, the sample will typically be in an aqueous solution. Sample solutions in other applications may include nonaqueous solutions. For instance, in food chemistry, methanolic solutions are used. The beads typically dissolve in less than about 30 seconds, preferably less than about 10 seconds, more preferably in less than about 1 second and in some cases less than about 0.3 seconds. In some case, the rapidity of dissolution gives the impression that the bead "explodes" and distributes the dissolving chemicals throughout the reconstituting volume. In other cases, the chemicals dissolve so rapidly that they are concentrated into the first fluid contacting the bead. Rapid dissolution of the beads is facilitated by a chemical lattice structure which quickly conducts water into the bead. To form the chemical lattice, fillers are included in the aqueous solution used to produce the beads. As the beads are lyophilized, these molecules facilitate formation of a network of open spaces or a chemical lattice in the beads. The filler components of the beads are typically polymeric compounds, such as bovine serum albumin, polyethylene glycol, dextran, Ficoll® (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.), or polyvinylpyrrolidone. In addition, emulsifiers such as sodium cholate and the like are useful as fillers. Monosaccharides and their derivatives, such as mannitol or the polyalcohol, myo-inositol, can also be used. Depending upon the particular application, the fillers can be used individually or in combination with one or more of the other filler components. In some cases, such as sodium fluoride and potassium oxalate, no filler is needed.

In addition to fillers, the beads of the present invention also comprise surfactants at concentrations sufficient to inhibit bubble formation when the beads are rapidly rehydrated. As described above, bubbles are detrimental to the assays because they interfere with optical measurements. If the beads comprise surfactants at the appropriate concentrations, however, such problems are avoided. Suitable surfactants include non-ionic detergents such as polyoxyethylene 9 lauryl ether, octoxynol 9, Synthrapol®, NP-90, Trycol® 5941, Trycols 6735 and the like. Ionic detergents such as Gafac® 560, sodium dodecyl sulfate and the like are also suitable. Typically, the surfactants are present in the reconstituted beads at a concentration between about 0.08 g and about 31 g per 100 ml. The surfactant concentration used will depend upon the particular reagent used in the assay.

The fillers and surfactants used in a particular dried bead preparation are preferably selected so as to minimize interference with the assay or other application. Optimization of the these components is facilitated by Table 1 which provides information regarding desired characteristics of fillers and surfactants suitable for use with reagents used in a variety of assays. In addition, the Example section below provides the precise concentrations of filler and surfactant components which have been found to be particularly useful in the exemplified assays.

In order to provide beads of the correct size in a test well, the components are typically concentrated in the bead. Upon rehydration with a predetermined volume of sample, the reagents and other components are present in the correct concentration. For instance, the components of the reagent beads for alkaline phosphate determinations are typically at about 6× concentration and total protein reagents are at about 2.7× concentration. In the case of sodium fluoride, it can be dispensed as a slurry of 12% (w/v). The ideal concentration for the reagents for particular assay can be easily determined, depending upon desired size of the bead, sample volume, and the like.

TABLE 1

(g/100 ml)

	fillers								surfactants					
	PEG 3400	PEG 8000	PEG 20M	Dextran	bovine albumin	PVP	inositol	mannitol	Mega 8	n-Octyl glucoside	Triton X-100	Trycol	Thesit	cholic acid
ALP A			5.40			0.10	1.0				0.08			
ALP B			5.40			0.10	1.0				0.08			
Amylase		4.00					2.0				0.30			
AST		2.50		2.50	2.50						0.40			
BUN		4.00									0.30			
Cholesterol (BMD)		0.87			3.70									
CRP			8.40								0.30			
Creatinine Test											0.21			2.0
Creatintine Blank											0.25			2.0
Glucose		1.80			2.10						0.30			
Plasma Dilution	6.0		1.00		2.00		1.0				0.38	2.10		10.0
Rotor Q.C.A	8.0		3.00								0.50			
Rotor Q.C.B	5.0		2.00				1.0				0.50			
Sample Blanking		9.60									0.80			
Temperature				1.00		0.10		10.00	0.20	0.20				
Bilirubin Enzyme				2.00			1.0	6.00						
Bilirubin Buffer		8.00												
Total Protein	5.00	4.00	0.50										0.143	0.5

TABLE 1-continued

(g/100 ml)

	fillers								surfactants					
	PEG 3400	PEG 8000	PEG 20M	Dextran	bovine albumin	PVP	inositol	mannitol	Mega 8	n-Octyl glucoside	Triton X-100	Trycol	Thesit	cholic acid
Triglycerides Blank		1.80		3.60	3.60						0.15	0.30		0.1
Triglycerides Test		1.80		3.60	3.60						0.15	0.30		0.1
Uric Acid		4.00									0.24			

The following examples show preparation of reagent beads for particular assays. These examples are provided by way of illustration and not by way of limitation.

EXAMPLE 1

Preparation of Reagent Beads for Total Protein Determination

The following solution was prepared by accurately weighing and dissolving the following chemicals in a container of about 800 ml of deionized or distilled water:

sodium potassium tartrate	37.80 g
sodium hydroxide pellets	28.20 g
cupric sulfate	12.00 g
potassium iodide	12.90 g
sodium carbonate	3.85 g
sodium cholate	5.00 g
polyoxyethylene 9 lauryl ether	1.43 g
polyethylene glycol (FW 3400)	50.00 g
polyethylene glycol (FW 8000)	40.00 g
polyethylene glycol (FW 20,000)	5.00 g

It is best to completely dissolve each chemical before adding the next chemical. After the last chemical dissolved, the solution volume was adjusted to 1.0 liter with deionized or distilled water. The solution was filtered through a stack of media that terminated in 0.45 micron porosity. The solution was then degassed using a vacuum pump. The above solution when diluted 37 ml plus 63 ml with water is used to assay Total protein concentration in various clinical samples such as serum or plasma. The sodium carbonate is added as a stabilizer, and polyoxyethylene 9 lauryl ether is added for controlling bubbles during dissolution. Sodium cholate and the various polyethylene glycols are added as fillers to facilitate formation of a chemical lattice during subsequent freeze drying.

The solution was dispensed by an IVEK model AAA pump in discrete 2.96 microliter drops at a rate of 1 to 2 drops-per-second. The discrete amounts of fluid drop through air, form beads and land on the surface of liquid nitrogen. The surface of the nitrogen does not need to be agitated. After freezing the beads were dried in Virtis freeze dryer (model no. 12EL console) (Gardener, N.Y.) until their residual moisture were less than 11% of the total remaining mass.

A freeze dried reagent bead prepared according to the above method can be reconstituted with 8 microliters of a mixture of water or diluent (14 parts) and human serum (1 part). The resulting change in absorbance at 550 nm minus the absorbance of a reagent bead reconstituted with 8 microliters of water or diluent and minus the absorbance of the human serum sample diluted in the same ratio with water plus polyethylene 9 lauryl ether and sodium cholate is proportional to the amount of total protein in the sample.

The imprecision (coefficient of variation) among the 1.78 millimeter diameter beads is:

dispensed frozen beads	1.5% at 3.7 mg
freeze dried beads	2.5% at 0.6 mg

Each reagent bead dissolves in 8 microliters of water or diluent within 5 seconds in a centrifugal analyzer.

EXAMPLE 2

Preparation of Reagent Beads for C-Reactive Protein Determination

The following solution was prepared by accurately measuring weighing and dissolving the following chemicals in a container of about 200 mls of deionized or distilled water:

C-reactive protein antibody	0.56 liters
Sodium chloride	25.50 g
HEPES	71.50 g
Triton ® X-100	3.00 g
polyethylene glycol (FW 20,000)	84.00 g

It is best to completely dissolve each chemical before adding the next chemical. After the last chemical dissolved, the pH was adjusted to 7.4 with dilute sodium hydroxide and the solution volume was adjusted to 1.0 liter with deionized or distilled water. The solution was filtered through a stack of media that terminated in 0.2 micron porosity. The solution was then degassed.

The above solution when diluted 33 ml plus 67 ml with water or diluent is used to assay C-reactive protein in various clinical samples such as serum or plasma. The sodium chloride is added as a stabilizer and Triton® X-100 is added for controlling bubbles during dissolution. Polyethylene glycol is added to facilitate the development of turbidity in the analytic reaction and as filler to facilitate formation of a chemical lattice during subsequent freeze drying.

The solution was dispensed by an IVEK model AAA pump in discrete 2.67 microliter drops at a rate of 1 to 2 drops-per-second. The discrete amounts of fluid drop through air, form beads and land on the surface of liquid nitrogen. The surface of the nitrogen does not need to be agitated. After freezing, the beads were dried in a Virtis freeze dryer (model no. 12EL console) until their residual moisture were less than 6% of the total remaining mass.

A freeze dried reagent bead prepared according to the above method can be reconstituted with 8 microliters of a mixture of water or diluent (14 parts) and human serum (1 part). The resulting change in absorbance at 340 nm minus the absorbance of a reagent bead reconstituted with 8 microliters of water or diluent and minus the absorbance of the human serum sample diluted in the same ratio with water

plus Triton® X 100 is proportional to the amount of C-reactive protein in the sample.

The imprecision (coefficient of variation) among the 1.72 millimeter diameter beads is:

dispensed frozen beads	1.7% at 2.9 mg
freeze dried beads	1.8% at 0.5 mg

Each reagent bead dissolves in 8 microliters of water or diluent within 3 seconds in a centrifugal analyzer.

EXAMPLE 3

Preparation of Reagent Beads for Alkaline Phosphatase (ALP) Determination

The following solutions were prepared. ALP part A: The following chemicals were accurately measured, weighed, and dissolved in a container of about 800 mls of deionized or distilled water:

Tris (hydroxymethyl) aminomethane-HCL	10.2 g
HEDTA	2.1 g
magnesium chloride hexahydrate	2.6 g
zinc sulfate heptahydrate	1.7 g
4-nitrophenylphosphate	35.6 g
polyethylene glycol (FW 20,000)	54.0 g
myo-inositol	10.0 g
Triton ® X-100	0.8 g
glycerol	6.0 g
polyvinylpyrrolidone (FW 30,000)	1.0 g

It is best to completely dissolve each chemical before adding the next chemical. After the last chemical dissolved, the pH was adjusted to 6.8 with dilute 2-amino-2-methyl-1-propanol and the solution volume was adjusted to 1.0 liter with deionized or distilled water. The solution was filtered through a stack of media that terminated in 0.2 micron porosity. The solution was then degassed.

ALP part B: The following chemicals were accurately measured, weighed, and dissolved in a container of about 800 mls of deionized or distilled water:

Tris (hydroxymethyl) aminomethane-HCL	10.2 g
Tris (hydroxymethyl) aminomethane	166.0 g
HEDTA	2.1 g
polyethylene glycol (FW 20,000)	54.0 g
myo-inositol	10.0 g
Triton ® X-100	0.8 g
2-amino-2-methyl-1-propanol	53.4 g
polyvinylpyrrolidone (FW 30,000)	1.0 g

It is best to completely dissolve each chemical before adding the next chemical. After the last chemical dissolved, the pH was adjusted to 10.3 with dilute 2-amino-2-methyl-1-propanol and the solution volume was brought to 1.0 liter with deionized or distilled water. The solution was filtered through a stack of media that terminated in 0.2 micron porosity. The solution was then degassed.

The above solutions when combined in equal volumes of 16.7 ml each and 67 ml of water or diluent are used to assay alkaline phosphatase in various clinical samples such as serum or plasma. The glycerol is added as a stabilizer. Triton® X-100 is added for controlling bubbles during dissolution. Polyethylene glycol, myo-inositol, and polyvinylpyrrolidone are added as fillers to facilitate formation of a chemical lattice during subsequent freeze drying.

The solutions were dispensed separately by an IVEK model AAA pump in discrete 2.67 microliter drops at a rate of 1 to 2 drops-per-second. The discrete amounts of fluid drop through air, form beads and land on the surface of liquid nitrogen. The surface of the nitrogen does not need to be agitated. After freezing the beads were dried in a Virtis freeze dryer (model no. 12EL console) until their residual moisture were less than 6% of the total remaining mass.

One of each, ALP A and ALP B, freeze dried reagent beads can be reconstituted with 16 microliters of a mixture of water or diluent (14 parts) and human serum (1 part). The resulting rate of change in absorbance at 405 nm is proportional to the amount of alkaline phosphatase in the sample.

The imprecision (coefficient of variation) among the 1.72 millimeter diameter beads is:

	ALP A	ALP B
dispensed frozen beads	0.4% at 2.8 mg	0.7% at 2.9 mg
freeze dried beads	1.5% at 0.5 mg	2.2% at 0.7 mg

The two reagent beads dissolve in 16 microliters of water or diluent within 10 seconds in a centrifugal analyzer. The active constituents in this assay are separated to improve reagent stability. One of each of the beads is placed in the same chamber for the ALP assay.

EXAMPLE 4

Preparation of Freeze-Dried Concentrated Potassium Reagent Containing Macrocyclic Ionophore Trinitroanilino Cryptahemispherand [2.2] for Potassium Determination

The active trinitroanilino cryptahemispherand [2.2] and surfactants (Brij® surfactants) were isolated from ChromoLytew Potassium Reagent (Technicon Instruments Corp., Tarrytown, N.Y. 10961) using Wide-Pore Butyl, 40 μM chromatographic medium (J. T. Baker Inc., Phillipsburg, N.Y. 08865) as follows:

25 g of Wide-Pore Butyl, 40 μM chromatographic medium were suspended in 360 ml of degassed isopropanol and then 360 ml of degassed deionized water was added. About 80% of the liquid was decanted. An additional 360 ml portion of degassed deionized water was added and the slurry in the flask was sonicated for two minutes, followed by two minutes of vacuum degassing. The suspended chromatographic medium was poured into an appropriately sized chromatographic column to form a 3–10 cm high packing bed. The packing was equilibrated by passing 250 ml of degassed deionized water through the column.

Five liters of ChromoLyte™ Potassium Reagent were applied to the column. The colored trinitroanilino cryptahemispherand [2.2] and the surfactants were adsorbed on the top of the column. The nonadsorbed triethanolamine buffer, also containing 3% of 2-(2-ethoxyethoxy)-ethanol (EEE) and stabilizers, was collected and saved for later use. The trinitroanilino cryptahemispherand [2.2] and the surfactants were eluted from the column with a mixture of previously degassed isopropanol (98%) and EEE (2%). The isopropanol was removed from the eluate using an evacuated rotary evaporator at room temperature to yield an oily, dark brown concentrate.

The previously collected buffer fraction was concentrated twofold using an evacuated rotary evaporator at 35°–40° C. The concentrated trinitroanilino cryptahemispherand [2.2], the surfactants and the remaining EEE were dissolved in 400 ml of the twofold concentrated buffer solution.

The following materials were measured, added and dissolved in the above solution:

polyethylene glycol (FW 3400)	40 g
isopropanol	5.0 ml
polyvinylpyrrolidone K-29-32	0.50 g

The pH of the solution was measured using an electrode pair with a calomel reference electrode to verify that the pH was less than 0.05 pH unit different from the pH of the starting ChromoLyte™ Potassium Reagent. If necessary, pH adjustment was made with a 20% triethanolamine solution or with a 20% triethanolamine HCl solution. Finally, the volume was adjusted to 500 ml with the twofold concentrated buffer solution. The reagent was filtered through a stock of media that terminated in 0.2 micron porosity. The preferred concentration of 2-(2-ethoxyethoxy)-ethanol is between about 3% and about 4.8%, and that of the polyethoxylauryl ether is between about 0.5 and about 1.0%.

The above solution when diluted 50 ml plus 50 ml with water or diluent is used to assay potassium in various clinical samples such as serum or plasma. The level of 2-(2-ethoxyethoxy)-ethanol is necessary to insure uniform freezing of the reagent and to aid in rapid resolubilization after freeze-drying. The isopropanol aids in creating the correct crystal structure during the freezing process so that the rehydration is facilitated. The Brij® surfactant (e.g., Brij® -35 or -58) aids in rehydration and in bubble inhibition. The polyethylene glycol is added to facilitate formation of a chemical lattice during subsequent freeze drying.

The solution was dispensed by an IVEK model AAA pump in discrete 4.0 microliter drops at a rate of 1 to 1.5 drops-per-second. The discrete amounts of fluid drop through air, form beads were dried in a Virtis freezer dryer (model no. 12 EL console) until their residual moisture were less than 6% of the total remaining mass. A freeze dried reagent bead prepared according to the above method can be reconstituted with 8 microliters of a mixture of water or diluent (14 parts) and human serum (1 part). The resulting change in absorbance at 500 nm minus the absorbance of a reagent bead reconstituted with 8 microliters of water or diluent and minus the absorbance of the human serum sample diluted in the same ratio with water plus Brij® surfactant is proportional to the amount of potassium in the sample.

The imprecision (coefficient of variation) among the 1.97 millimeter diameter beads is:

dispensed frozen beads	1.5% at 2.6 mg
freeze dried beads	1.6% at 0.5 mg

Each reagent bead dissolves in 8 microliters of water or diluent within 5 seconds.

EXAMPLE 5

Preparation of Sodium Fluoride Beads

The following solution was prepared. To a volumetric flask containing 85 ml of deionized water was added 12.0 g of sodium fluoride. The volume was than adjusted to 100.0 ml with additional deionized water. The solution was vigorously mixed until a uniform slurry was formed. The slurry was continuously mixed during dispensing by an IVEK model AAA pump in 6.0 ul drops at a rate of 1 or 2 drops per second. The discrete amounts of fluid drop through air, form beads and land on the surface of liquid nitrogen. After freezing the beads were dried in a Virtis freeze dryer (model no. 12EL console). Seven beads of sodium fluoride are required to arrest glycolysis per 2 ml of blood.

The Imprecision (coefficient of variation) among the 2.25 millimeter diameter beads is:

dispensed frozen beads	0.6% at 3.0 mg
freeze-dried beads	2% at 0.5 mg

Seven beads dissolve in 2.0 ml of blood within 10 seconds. with a single inversion of the container to mix the sodium fluoride with the blood.

EXAMPLE 6

Preparation of Potassium Oxalate Beads

The following solution was prepared. To a volumetric flask containing 75 ml of deionized water was added 20.0 g of potassium oxalate. The volume was then adjusted to 100.0 ml with additional deionized water. The solution was vigorously mixed until dissolved. The solution was continuously mixed during dispensing by an IVEK model AAA pump in 20.0 ul drops at a rate of 1 to 2 drops per second. The discrete amounts of fluid drop through air, form beads and land on the surface of liquid nitrogen. After freezing the beads were dried in a Virtis freeze dryer (model no. 12EL console). One bead of potassium oxalate is required to prevent coagulation per 2 ml of blood.

The Imprecision (coefficient of variation) among the 3.37 millimeter diameter beads is:

dispensed frozen beads	0.4% at 21 mg
freeze-dried beads	0.7% at 2.5 mg

One bead dissolves in 2.0 ml of blood within 10 seconds, with a single inversion of the container to mix the potassium oxalate with the blood.

The above examples illustrate preparation of particular reagent beads within the scope of the present invention. The examples have been provided for the purposes of clarity and understanding the invention. It will be apparent, however, that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A container holding a dried chemical composition which dissolves in less than about 10 seconds in water, wherein said dried chemical composition comprises a preselected precisely measured aliquot of said dried chemical composition which chemical composition is in bead form have in a diameter between 1.5 mm and 10.0 mm.

2. The container of claim 1, which is a cuvette in a centrifugal rotor.

3. The container of claim 1, wherein the dried chemical composition has a diameter between about 1.5 mm and 3.5 mm.

4. The container of claim 1, wherein said dried chemical composition is produced by a method comprising the steps of:

forming a solution comprising a desired compound;

dispensing uniform, precisely measured drops of the solution into a cryogenic liquid, whereby the drops are frozen; and

drying the frozen drops, thereby forming dried aliquots wherein the dried aliquots comprise a plurality of dried aliquots having a coefficient of weight variation of less than about 3 % and wherein said dried chemical composition is a single aliquot selected from the plurality of dried aliquots.

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APPENDIX C: RELATED PROCEEDINGS

No related proceedings are pending.